Evaluating the Efficacy of Transcranial Temporal Interference Stimulation (tTIS) in Humans

Thesis

for the degree of

doctor rerum naturalium (Dr. rer. nat.)

approved by the Faculty of Natural Sciences of Otto von Guericke University Magdeburg

by M. Sc. Carsten Thiele

born on 21.12.1990 in Emden

Examiner: Prof. Dr. Tino Zähle

Prof. Dr. Bettina Schwab

Submitted on: 06.11.2024

Defended on: 31.03.2025

Table of Contents

List of Figures

List of Abbreviations

- BOLD - Blood-Oxygen-Level-Dependent
- CNS – Central Nervous System
- DBS - Deep Brain Stimulation
- EEG - Electroencephalography
- GABA - Gamma-Aminobutyric Acid
- IFC - Interferential Current Therapy
- LFP - Local Field Potential
- LTP - Long-Term Potentiation
- M1 - Primary Motor Cortex
- MEP - Motor Evoked Potential
- MRI - Magnetic Resonance Imaging
- NSE - Neuron-Specific Enolase
- PWM-TI - Pulse-Width Modulated Temporal Interference
- SEEG - Stereo-Electroencephalography
- SLE - Seizure-Like Event
- tACS - Transcranial Alternating Current Stimulation
- tES - Transcranial Electrostimulation
- tTIS - Transcranial Temporal Interference Stimulation
- V/m - Volts per meter
- Δf - Frequency Difference

Abstract

Transcranial Temporal Interference Stimulation (tTIS) is an emerging non-invasive brain stimulation technique that offers the potential to target deeper brain areas with high focality, overcoming limitations of traditional electrostimulation methods such as transcranial Alternating Current Stimulation (tACS). Empirical evidence supporting its efficacy has primarily been obtained from a limited number of murine studies, with even fewer conducted in humans. This dissertation aims to address this gap by providing a comprehensive exploration of tTIS in humans. It seeks to provide proof-of-concept evidence, establishing the feasibility and efficacy of tTIS, thereby unlocking potential applications in both research and therapeutic settings.

Study 1 examined whether tTIS could induce neuronal activation in retinal cells, using phosphenes as markers of stimulation efficacy. Although phosphenes were not induced, the study provided valuable insights into the neuronal activation capabilities of tTIS, suggesting that neuronal modulation might be a more promising avenue.

Based on these findings, Study 2 investigated the effects of tTIS at alpha frequencies to modulate activity in parieto-occipital regions. The results demonstrated significant changes in event-related desynchronization (ERD) during a mental rotation task, indicating entrainment of alpha oscillations during task performance. Notably, the study found no stimulation effect on alpha activity at rest, highlighting the necessity of an active neuronal network for effective modulation.

Study 3 addressed a key limitation of current tTIS applications: the lack of exploration of stimulation intensities beyond 2 mA, which may constrain efficacy. To overcome this limitation, the study investigated higher stimulation intensities for human use, demonstrating that up to 4 mA could be safely and comfortably administered.

In summary, this dissertation establishes tTIS as a promising technique for non-invasive brain stimulation, capable of modulating activity in engaged neuronal networks. These findings provide a strong foundation for future research and potential clinical applications, paving the way for advanced neurotherapeutic interventions.

German Abstract (Zusammenfassung)

Die transkranielle temporale Interferenzstimulation (tTIS) ist eine aufstrebende, nicht-invasive Hirnstimulationstechnik, die das Potenzial bietet, tiefere Hirnareale mit hoher Fokalität zu erreichen und damit die Einschränkungen herkömmlicher Elektrostimulationsmethoden wie der transkraniellen Wechselstromstimulation (tACS) zu überwinden. Empirische Belege für ihre Wirksamkeit stammen bislang nur aus einer begrenzten Anzahl von Studien an Mausmodellen, während Untersuchungen am Menschen noch seltener sind. Diese Dissertation zielt darauf ab, die Lücke in humanen In-vivo-Studien zu schließen, indem sie eine umfassende Erforschung von tTIS beim Menschen bietet. Sie strebt einen Machbarkeitsnachweis an und etabliert die Wirksamkeit von tTIS, um weitere potenzielle Anwendungen in Forschung und Therapie zu ermöglichen.

Studie 1 untersuchte, ob tTIS eine neuronale Aktivierung in retinalen Zellen induzieren kann, wobei Phosphene als Marker für die Stimulationswirksamkeit dienten. Obwohl keine Phosphene induziert wurden, lieferte die Studie wertvolle Einblicke in die neuronalen Aktivierungsmöglichkeiten von tTIS und deutete darauf hin, dass die neuronale Modulation ein vielversprechenderer Ansatz sein könnte.

Aufbauend auf diesen Erkenntnissen erforschte Studie 2 die Effekte von tTIS bei Alphafrequenzen zur Modulation der Aktivität in parieto-okzipitalen Regionen. Die Ergebnisse zeigten stimulationsspezifische Veränderungen in der ereigniskorrelierten Desynchronisierung (ERD) während einer mentalen Rotationsaufgabe, was auf eine Veränderung von Alpha-Oszillationen während der Aufgabenbearbeitung hindeutet. Bemerkenswerterweise wurde kein Stimulationseffekt auf die Alpha-Aktivität in Ruhe festgestellt, was die Notwendigkeit eines aktiven neuronalen Netzwerks für eine effektive Modulation unterstreicht.

Studie 3 adressierte eine zentrale Einschränkung aktueller tTIS-Anwendungen: das Fehlen von Untersuchungen zu Stimulationsintensitäten über 2 mA hinaus, was die Wirksamkeit einschränken

könnte. Um diese Limitierung zu überwinden, untersuchte die Studie höhere Stimulationsintensitäten für den menschlichen Gebrauch und zeigte, dass bis zu 4 mA sicher und komfortabel verabreicht werden können.

Zusammenfassend etabliert diese Dissertation tTIS als eine vielversprechende Technik für die nicht-invasive Hirnstimulation, die in der Lage ist, die Aktivität in aktiven neuronalen Netzwerken zu modulieren. Diese Ergebnisse bilden eine solide Grundlage für zukünftige Forschung und potenzielle klinische Anwendungen und ebnen den Weg für fortschrittliche neurotherapeutische Interventionen.

1. General Introduction

1.1. The Evolution of Brain Stimulation Concepts

The understanding of the brain's role in human physiology and cognition has evolved significantly across millennia, from ancient philosophies to modern neuroscience. In ancient Greece, the prevailing view was that the brain merely cooled the blood, while the heart was considered the center of intelligence (Lloyd 1975). Hippocrates' theory of the Four Humors attributed personality traits and emotional states to a balance of four bodily fluids: blood, yellow bile, black bile, and phlegm, further diminishing the perceived role of the brain (Hankinson 1998). In the 18th century, Benjamin Franklin's "fluid theory" of electricity proposed that electrical charge resulted from a surplus or deficit of a single ethereal fluid (Fowler 1997), laying the groundwork for later understandings of electrical phenomena. Today, our understanding of the brain as the seat of consciousness and the central controller of all bodily functions is well-established. Modern science has revealed that the brain's electrical activity is driven by chemoelectrical processes involving the flow of ions along neural pathways (Buzsáki et al. 2012). With this deepened understanding of the brain's mechanisms, our ability to probe and influence its functions has greatly improved. This relationship between understanding and manipulation is reciprocal and longstanding. A popular historical example involves the roman physician Scribonius Largus, who treated headaches by placing a torpedo fish, capable of delivering electric shocks, on his patients' heads (Francis and Dingley 2015). Interestingly, Scribonius' concept parallels modern electroconvulsive therapy, which involves administering strong electric currents to a patient’s head to treat treatment-resistant depression, though it is now performed under anesthesia and with muscle relaxants (Abrams 1989; Read and Bentall 2010; Espinoza and Kellner 2022).

1.2. Non-Invasive Stimulation Techniques: From tES to tTIS

Since the turn of the millennium, research has increasingly focused on less invasive methods such as non-invasive transcranial electrostimulation (tES) (Nitsche and Paulus 2000, 2001; Antal et al. 2003). This technique involves attaching electrodes to the scalp and applying a mild electric current, typically no more than 2 mA, which may cause slight tingling or itching sensations (Fertonani et al. 2015). This weak current modulates, but does not induce, neuronal activity in the affected brain regions beneath (Khadka and Bikson 2022; Reed and Cohen Kadosh 2018; Antal et al. 2008). A common form of tES is transcranial alternating current stimulation (tACS) (Antal and Paulus 2013; Antal et al. 2008), where an alternating current at a specific frequency is administered to the scalp. This technique leverages the principle that neural communication in the brain operates through rhythmically oscillating neuronal activity (Berger 1929; Thut et al. 2012; Jacobs et al. 2007). The externally applied current acts as a pacemaker, phase-locking internal oscillatory activity (Reato et al. 2010; Thut et al. 2011; Herrmann et al. 2013). This principle is known as neuronal entrainment (Vogeti et al. 2022; Pikovsky et al. 2001). For instance, applying an alternating current at the alpha frequency to the parieto-occipital area can facilitate its alpha rhythm (Zaehle et al. 2010; Ruhnau et al. 2016; Huang et al. 2021). Consequently, tACS has been used in various studies to effectively tune the brain's natural frequencies to desired effects. For example in studies researching attention (Schuhmann et al. 2019), perception (Rufener et al. 2016; Marchesotti et al. 2020), working memory (Röhner et al. 2018; Hoy et al. 2015; Pahor and Jaušovec 2018) or motor performance (Lasbareilles et al. 2023; Bieler; Miyaguchi et al. 2019). In clinical studies, tACS is being used to aid in treating depression (Alexander et al. 2019; Riddle et al. 2020), stroke (Naros and Gharabaghi 2017; Meng et al. 2024), and dyslexia (Rufener and Zaehle 2021) among others (Elyamany et al. 2021).

However, a significant limitation of current tES methods is their inability to effectively target deeper brain areas due to the rapid attenuation of current intensity with increasing depth. While this attenuation

could potentially be counteracted by increasing the stimulation intensity manyfold, doing so would lead to strong, undesired co-activation of overlying cortical regions (Bland and Sale 2019). To date, the only reliable method to stimulate deeper brain regions is through the invasive procedure known as deep brain stimulation (DBS) (Ashkan et al. 2017; Lozano et al. 2019). This technique involves the stereotactic surgical implantation of electrodes directly into the target brain region. In humans, DBS is used to treat ailments caused by abnormal or disrupted neural activity in the brain. A prominent example is the Parkinson's disease, which is characterized by the progressive loss of dopamine-producing neurons in the substantia nigra (Volkmann 2004; Ramirez-Zamora and Ostrem 2018; Limousin and Foltynie 2019). The absence of dopamine, a crucial neurotransmitter for regulating movement and coordination, leads to significant motor control disruptions in Parkinson's disease, manifesting as rigidity, tremors, and balance issues. One of the potential targets for DBS in Parkinson's disease is the subthalamic nucleus (STN). Dopamine normally regulates the activity of the STN; when dopamine levels are depleted, the STN becomes overactive, contributing to these motor symptoms. DBS mitigates these symptoms by delivering high-frequency electrical stimulation through electrodes implanted in the STN, inhibiting its overactivity and helping restore normal motor network function. This can significantly improve symptoms and enhance the overall quality of life for patients.

To overcome the limitations of current tES methods in targeting deep brain regions without undesired activation of superficial cortical areas, a recent breakthrough in neuroscience combines the deep stimulation capabilities of DBS with the non-invasiveness of tES. Transcranial Temporal Interference Stimulation (tTIS) addresses this challenge by focusing stimulation on deeper brain regions through the interference of high-frequency currents, thereby minimizing activation of overlying cortical tissue. Unlike current tES methods, which apply a single electric field, tTIS utilizes two simultaneously applied electric fields (see Figure 1). These fields propagate through the brain and intersect at the targeted region. The functionality of tTIS hinges on the use of alternating currents at high frequencies,

typically exceeding 1000 Hz, with a minor frequency difference Δf between them (e.g., $\Delta f = f_1 - f_2$ = 1010 Hz – 1000 Hz = 10 Hz). Importantly, this high frequency, or carrier frequency, is deliberately set above the neurons' modulation threshold (Hutcheon and Yarom 2000). Therefore, this carrier frequency does not modulate neuronal activity, as neurons exhibit a low-pass filtering characteristic, which prevents them from responding to high frequencies beyond this biological cutoff. This limitation stems from the biomechanical properties of the neuronal cell membrane, which regulates ion exchange at a finite rate that cannot indefinitely increase. This leads to the crucial fact that the driving stimulation factor is instead the low-frequency offset Δf between both electric fields, that results in neuronal modulation via entrainment. The underlying mechanism involves the inherent phase misalignment between the two fields, due to their frequency difference, which results in an amplitude-modulated signal at the envelope (or "beat") frequency in the specific region where both electric fields overlap. This modulation occurs because of alternating constructive and destructive interference; When both fields share the same polarity—either both positive or negative—the amplitude of the combined field increases through constructive interference. Conversely, destructive interference occurs when the fields have opposite polarities—one positive and one negative—resulting in a decreased amplitude of the field sum. This oscillating pattern of increasing and decreasing stimulation amplitudes is believed to modulate neuronal activity in a manner similar to tACS via neuronal entrainment.

Crucially, the locus of stimulation—the area where the superposition of both electric fields is greatest, leading to the most significant interference and thus the most pronounced amplitude-modulated waveform—is where the most effective stimulation occurs. This locus can be controlled through (1) the placement of the electrodes and (2) the current ratios of the electric fields. While the impact of electrode placement on the distribution of electric fields is relatively straightforward, adjusting current ratios influences the locus of stimulation through the dynamics of interference, specifically by altering the amplitude modulation of the resulting signal.

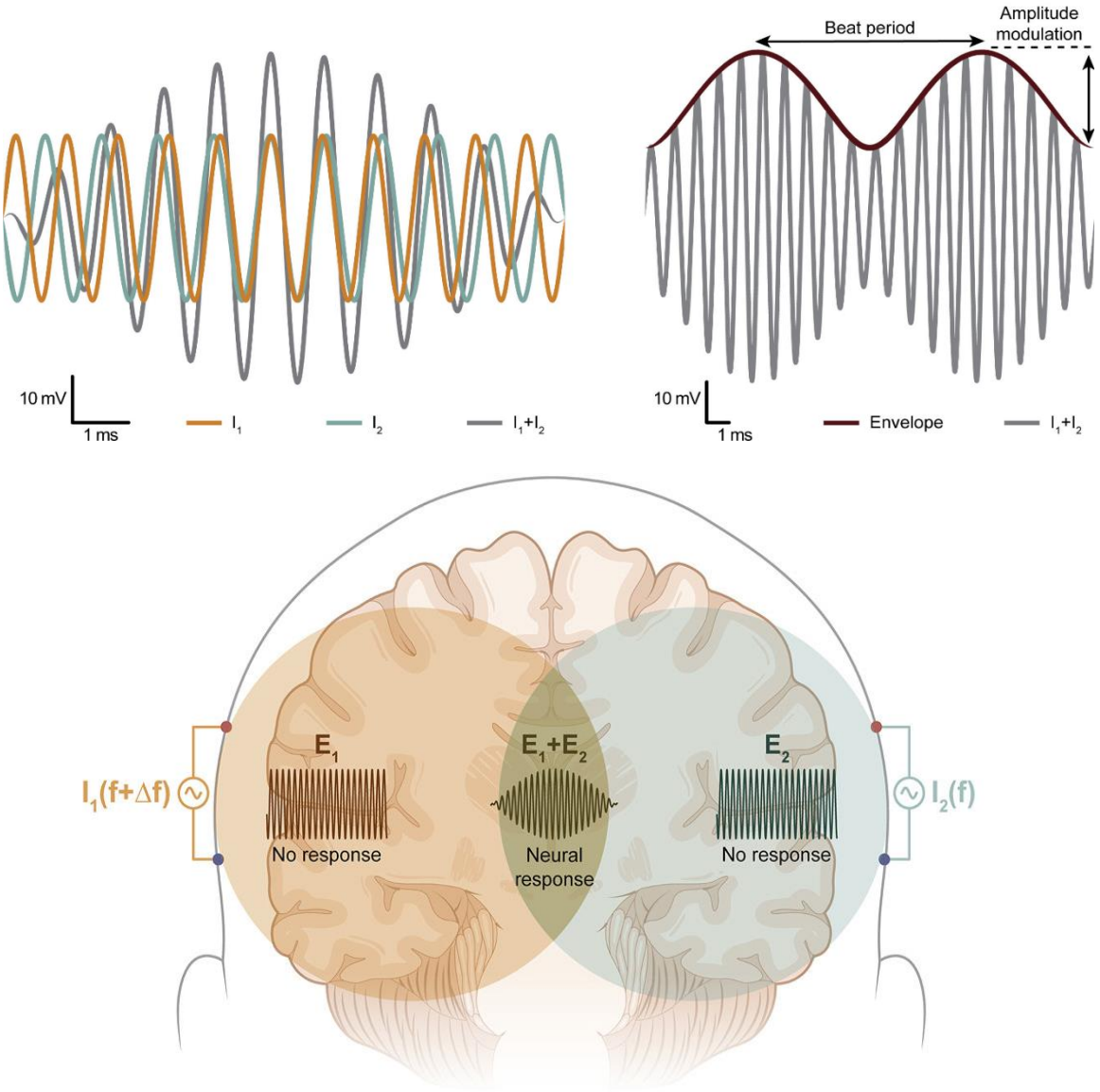

Figure 1 | The underlying principle of tTIS. **(Top Left)** Two electric fields (I_1 and I_2) with a frequency offset Δf (e.g. $\Delta f = f_1 - f_2 = 1010$ Hz – 1000 Hz = 10 Hz) are simultaneously applied. In the brain, these electric fields interfere, generating an amplitude modulated waveform ($I_1 + I_2$) via constructive and destructive interference. When both fields have the same polarity at a given time point, constructive interference increases the amplitude of the combined field. Conversely, at time points where the polarities are opposite, destructive interference reduces the amplitude of the combined field. **(Top Right)** The amplitude of the combined electric field oscillates at the "beat" frequency (Δf), generating an envelope, or amplitude-modulated waveform, that varies in strength over time. Here, the envelope represents the outer bounds of the fluctuating waveform and defines the degree of amplitude variation. **(Bottom)** The amplitude-modulated signal is strongest at the point of equal intensity of both individual fields. As the individual fields do not themselves exert a neuromodulatory effect, but only the amplitude-modulated signal, this effectively limits the stimulated region to this area. This causes (1) a high focality of the method and (2) the possibility to guide the locus of stimulation into deeper brain regions. *Note.* Taken from Mirzakhalili et al. (2020) and reprinted under Creative Commons CC.

The amplitude modulation refers to the extent of variation in the amplitude of the combined electric fields due to interference, essentially quantifying how much the amplitude fluctuates between its maximum and minimum values. The strongest constructive and destructive interference occurs where the intensities of both electric fields are equal, resulting in maximal amplitude modulation, which can induce a more pronounced neuronal response. Conversely, if the electric fields are unequal in intensity, a lower amplitude modulation is yielded (see **Figure 2**).

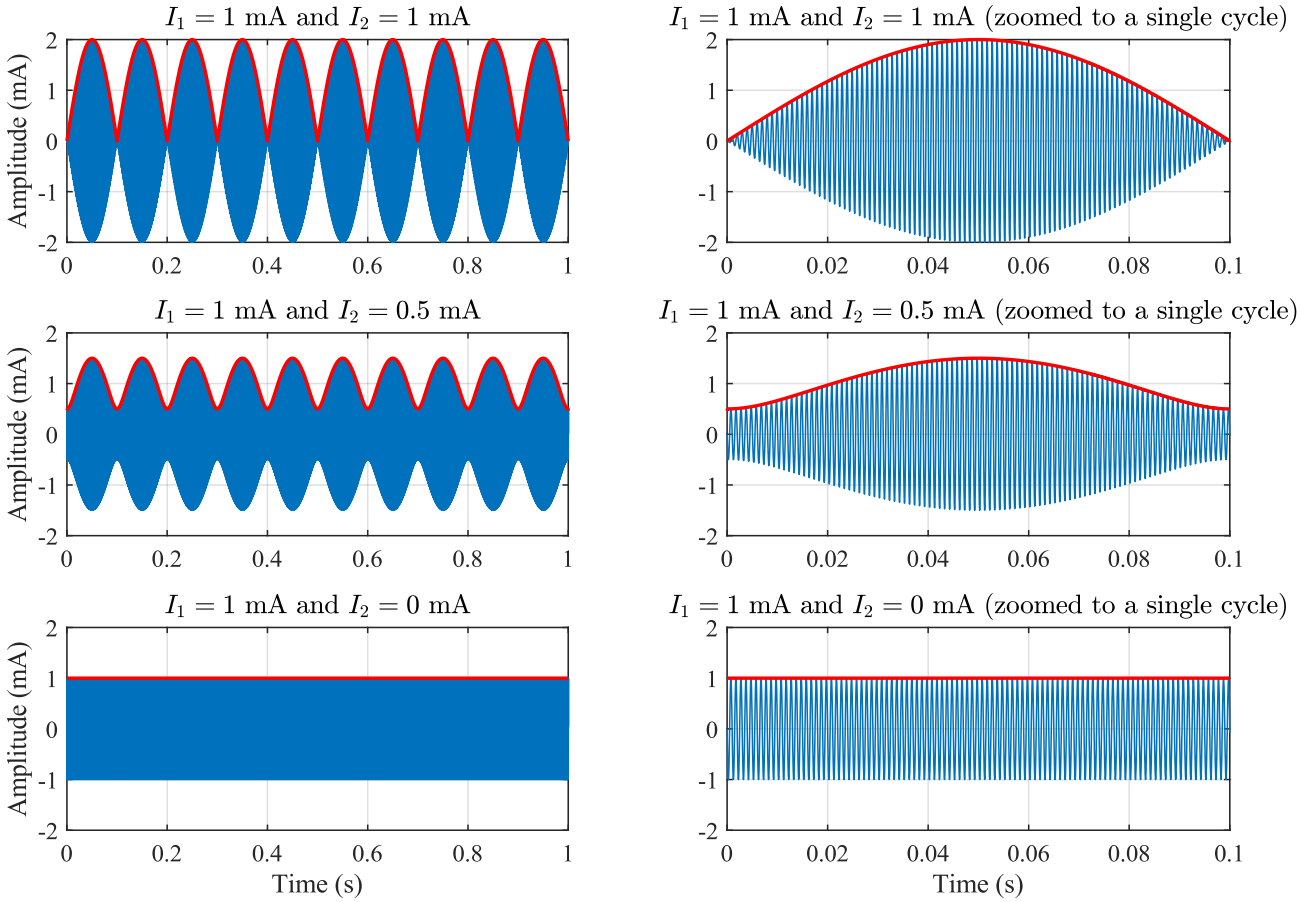

Figure 2 | Visualization of different amplitude modulations. This figure illustrates how constructive and destructive interference between two alternating currents (frequencies f_1 = 1000 Hz and f_2 = 1010 Hz) results in an amplitude-modulated waveform. The blue wave represents the resultant waveform, while the red line depicts the amplitude modulation, which corresponds to the strength of the stimulation effect. **(Top row)** When both fields are at equal intensity (I_1 = 1 mA, I_2 = 1 mA), the amplitude modulation is maximal. **(Middle row)** When one field is weaker than the other (I_1 = 1 mA, I_2 = 0.5 mA), interference effects are reduced, leading to a lower amplitude modulation and a correspondingly reduced stimulation effect. **(Bottom row)** When the intensity of the second field is zero (I_1 = 1 mA, I_2 = 0 mA), there is no interference or amplitude modulation, as only a single current is present; consequently, there is no stimulation effect. The right column zooms in on a single cycle under each condition.

It follows that the region where both electric fields are equal in intensity generates the greatest amplitude modulation and, therefore, the strongest stimulation effect. When equal intensities are applied, the strongest amplitude-modulated signal is located at the midpoint between the electric fields. Conversely, by reducing the intensity of one field, the stimulation locus shifts toward the electrode with the lesser intensity without the need to reposition the electrodes.

Notably, Acerbo et al. (2022) confirmed the confinement of the stimulation locus to the interference area, i.e., where both fields overlap. In this study, stereo-electroencephalography (SEEG) electrodes were implanted into a human cadaver's skull to measure electric current propagation. They demonstrated that the amplitude modulated signal peaked in the targeted hippocampal area and rapidly diminished outside this region. This is also in line with a similar cadaver study by Violante et al. (2023), who reached the same conclusions. Similarly, Liu et al. (2024) used SEEG electrodes implanted in live primates to confirm that the amplitude-modulated signal was generated in and confined to the targeted area.

Simulation tools such as SimNibs (Puonti et al. 2020; Thielscher et al. 2015) and ROAST (Huang et al. 2019) can be utilized to model the distribution of current densities in the brain. A recent SimNibs update now simulates the strength of amplitude-modulated waveforms when two electric fields are simultaneously applied, enabling predictions about how tTIS electrode placements and chosen current ratios will change the electric field strength delivered to specific areas. This opens the possibility to optimize stimulation parameters and achieve high current densities in target regions. Notably, this is only a forward-solution from chosen electrode setup to resulting electric field strength in the target region. Currently, more sophisticated models are being developed which allow for the inverse solution, where a target region can be declared, and the optimized electrode setup is generated.

To explore the biophysics behind tTIS, Esmaeilpour et al. (2021) computed the effect of tTIS on a neural network model. Their findings indicate that the efficacy of tTIS in modulating neuronal activity hinges on a rapid membrane time constant, suggesting that axonal sodium ion channels with their rapid time constants (Cao et al. 2020) are likely the primary mediators of the stimulation. Mirzakhalili et al. (2020) substantiate these claims, as they discovered that through the process of signal rectification, the amplitude modulated signal is demodulated at the axon's nodes of Ranvier to extract its low-frequency component (see **Figure 3**). This rectification is made possible due to nonlinear dynamics inherent to ion channel behavior. Specifically, tTIS is theorized to induce a strong influx of sodium ions, which exceeds the induced efflux of potassium ions, leading to a depolarization of the axon. In addition, the unique gating properties of sodium channels, which activate more rapidly than they inactivate, facilitate

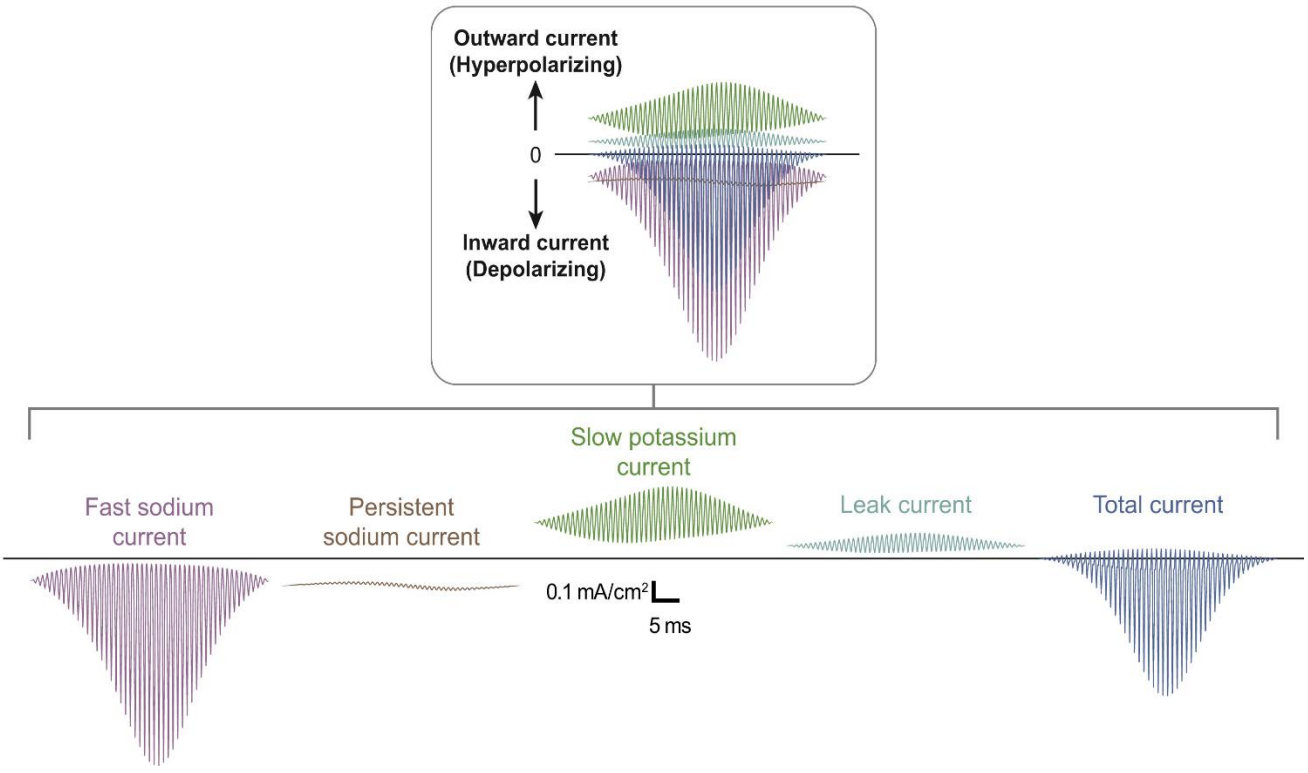

Figure 3 | Biophysics of tTIS. TTIS induces an exchange of ions at the axon, specifically at the node of Ranvier. Axonal sodium ion channels, due to their fast time constant, cause a strong inflow of sodium ions into the axon, which is more pronounced than the outflow of potassium through its slower ion channels. This results in a net positive current into the axon, leading to depolarization of the membrane. *Note.* Taken from Mirzakhalili et al. (2020) and reprinted under Creative Commons CC BY NC ND license.

the depolarization. This, in sum, leads to the activation of Gamma-Aminobutyric Acid ($GABA_b$) mediated inhibitory synapses (Esmaeilpour et al. 2021), causing a modulation of the neuronal network activity. However, the study by Mirzakhalili et al. (2020) introduces the possibility of a potential conduction block in these off-target areas, suggesting that neuronal activity in these regions might be unintentionally inhibited. Though their study suggests that this is mainly the case using very high stimulation intensities. As of today, further research is needed to confirm this possibility of a conduction block.

Regarding questions about the efficacy of tTIS, the study by Negahbani et al. (2018) utilized computational models to simulate the effects of an amplitude-modulated waveform, as is generated by tTIS. This approach was compared against conventional low-frequency tACS. Their findings effectively demonstrated phase-locking between local field potentials (LFPs) and the applied stimulation frequency, confirming successful entrainment of both pyramidal and fast-spiking inhibitory cells under both stimulation paradigms. This synchronization suggests that amplitude-modulated waveforms, much like traditional tACS, are capable of modulating neural activity through similar entrainment mechanisms, highlighting its potential in neural modulation. As of today, this has been further substantiated by other computational studies, theoretically confirming tTIS's efficacy (Rampersad et al. 2019; Karimi et al. 2019; Su et al. 2021; Howell and McIntyre 2021; Gomez-Tames et al. 2021; Wang et al. 2022; Albarran 2023).

1.3. Current Research and Safety Considerations in tTIS

Empirically, the seminal study by Grossman et al. (2017) was the first to report evidence of tTIS's efficacy *in-vivo* in rodents. This study demonstrated (1) the induction of motor activity in the extremities through motor cortex stimulation and (2) the induction of c-fos expression in hippocampal slices after stimulation (see **Figure 4**). It was only some years later that more rodent studies were

published demonstrating tTIS's efficacy to, for example, restore breathing after an opioid overdose (Sunshine et al. 2021), suppress epileptic biomarkers (Acerbo et al. 2022), enhance motor performance (Liu et al. 2023), induce eye- (Song et al. 2021) or paw movements (Zhang et al. 2022b) among others. Interestingly, Vieira et al. (2023) were able to even demonstrate tTIS ability in-vivo in primates by disrupting spike timings during a visual fixation task.

Notably, the concept behind tTIS, as explored by Grossman et al. (2017), was not entirely novel. Previously, a similar technique known as interferential current therapy (IFC) has been explored (Ward 2009; Beatti et al. 2011; Goats 1990). However, IFC has primarily been used to stimulate peripheral nerves and muscle tissue (Waza et al. 2017; Albornoz-Cabello et al. 2019), whereas tTIS targets the modulation of the central nervous system (CNS).

Safety is paramount for any new method intended for human use, and tES methods are well-validated as safe. Liebetanz et al. (2009) found in rodent studies that tissue damage from stimulation only occurred at intensities of at least 142.9 A/m² applied for 10 minutes, equivalent to a charge density of 52400 C/m². In contrast, typical human tES studies use up to 2 mA for 20 minutes with electrode sizes of 5x5 cm, resulting in a charge density of 960 C/m², which is significantly below the threshold associated with any risk. This high safety margin is supported by further studies, such as Jackson et al. (2017) and Zhang et al. (2019), which reported no lesions at charge densities used in human protocols. Additional human studies reinforce this safety profile; for instance, Nitsche et al. (2003) observed no increase in serum levels of neuron-specific enolase (NSE) — a marker of neuronal damage — post-stimulation. Follow-up research by Nitsche et al. (2004) confirmed that tES does not induce brain edema, blood-brain barrier alterations, or cerebral tissue changes detectable by MRI. Moreover, Tadini et al. (2011) reported no adverse effects beyond mild headaches, which were equally prevalent in both sham and active stimulation groups, suggesting they were not attributable to the stimulation. This evidence has led to the consideration that using up to 4 mA in tES is safe (Bikson et al. 2016;

Matsumoto and Ugawa 2017; Fertonani et al. 2015). Still, for tTIS, the use of high-frequency alternating currents introduces new considerations that require specific safety evaluations. Initial assessments were considered by Grossman et al. (2017), who conducted a comprehensive safety evaluation on rodents and found no immunohistochemical signs of tissue damage, supported by measurements showing no significant tissue heating. Further assessing tTIS safety in humans, Cassarà et al. (2022) applied tTIS at 2 mA to 38 healthy volunteers, finding no significant differences in serum levels of NSE between sham and active stimulation groups. These findings are in line with a similar study by Piao et al. (2022). These studies collectively suggest that tTIS can be considered as safe for human use.

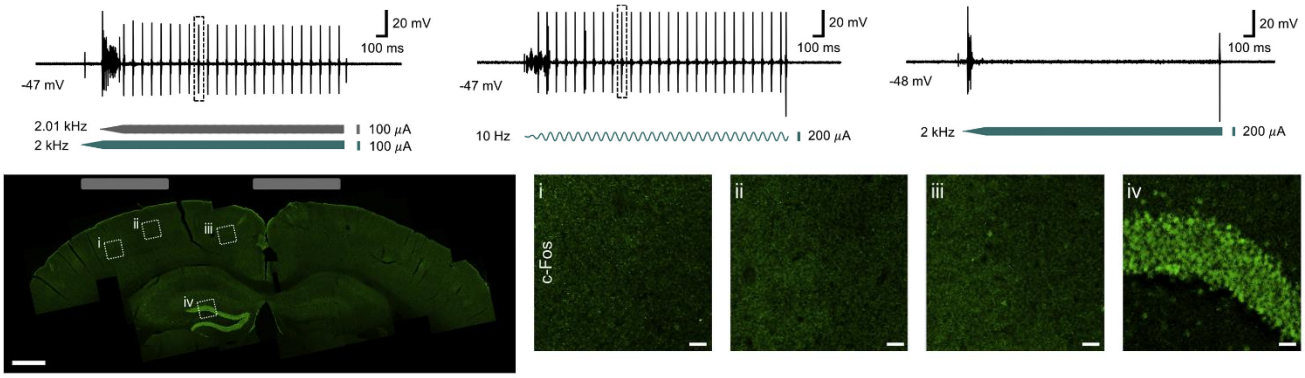

Figure 4 | TTIS effects on neural activity in rodents. **(Top Left)** In-vivo whole-cell patch clamp recordings during tTIS. The application of two high-frequency alternating current electric fields, with f_1 = 2.01 kHz and f_2 = 2 kHz, results in an amplitude-modulated signal with a 10 Hz envelope. Cell recordings demonstrate an entrainment of neural activity in sync with the applied 10 Hz amplitude-modulated signal. **(Top Middle)** Cell patch clamp recordings during the application of a 10 Hz alternating current, equivalent to using the well-established tACS as a stimulation method. The recordings show the typical entrainment effect of tACS, with neural activity synchronized to the stimulation frequency. Additionally, the stimulation effects of tTIS and tACS do not differ, suggesting comparable efficacy for both methods. **(Top Right)** Recordings during the application of a 2 kHz alternating current. As no amplitude-modulated signal is generated, neurons are unresponsive, demonstrating that the individual high-frequency fields do not exert a stimulation effect on the neurons. **(Bottom Row)** C-Fos staining of slices taken from a stimulated mouse brain. The target area for stimulation was the hippocampus. Slices (i), (ii), and (iii) do not express c-FOS, indicating no stimulation effect in terms of increased neuronal activation in the off-target areas. Slice (iv) shows strong c-Fos expression, suggesting significant neuronal activation in the target area. *Note.* Taken from Grossman et al. (2017) and reprinted under Creative Commons CC-BY license.

1.4. Aims of this Thesis

When this thesis was begun in November 2019, no in vivo human studies were available, despite the promising potential of tTIS for treating neurological and psychiatric conditions as suggested by Grossman et al. (2018). Today, while some studies have been conducted, research on tTIS in humans remains limited. Among the earliest, Ma et al. (2021) applied tTIS to the motor cortex of 21 participants and were able to observe improvements in a motor task, suggesting enhanced motor learning. They also noted increased brain excitability, as indicated by increased amplitudes of motor evoked potentials (MEPs). In another study, Zhu et al. (2022) demonstrated increased functional connectivity between the primary motor cortex and secondary motor areas using functional magnetic resonance imaging (fMRI). Zhang et al. (2022a) attempted to enhance working memory performance with tTIS applied to frontoparietal areas but did not find a significant advantage over sham stimulation. Similarly, Conta et al. (2022) explored the impact of tTIS on parieto-occipital alpha oscillations but found no significant increase in alpha activity compared to an active sham condition. A similar study by Iszak et al. (2023) also failed to demonstrate parieto-occipital alpha entrainment in their pilot study. In contrast, Violante et al. (2023) reported that tTIS increased hippocampal activity, evidenced by heightened BOLD activity and improved performance in a visual episodic memory task. Missey et al. (2023) reported using tTIS for hypoglossal nerve stimulation to alleviate symptoms of obstructive sleep apnea by preventing the tongue from collapsing into the airway. Lastly, Zheng et al. (2024) demonstrated that applying tTIS to the primary motor cortex to target lower limb motor control effectively enhanced training outcomes. Participants in the tTIS group exhibited significantly greater improvements in jumping height compared to those in a sham group. Although these findings are promising, the overall body of evidence for tTIS efficacy in humans remains limited.

Despite this limited evidence base, tTIS holds significant potential for advancing both our understanding and application of non-invasive brain stimulation. However, the field remains largely in

the proof-of-concept stage, especially in human applications. This thesis aims to contribute to the growing knowledge of tTIS by providing a comprehensive exploration of its feasibility, efficacy, and potential applications in humans.

2. General Discussion

2.1. Summary

tTIS is an innovative approach in non-invasive brain stimulation techniques, offering the advantage of precisely targeting deeper brain areas without affecting surrounding regions. This technique has the potential to unlock new possibilities in therapeutic treatments and research paradigms (Grossman et al. 2018). Following the pioneering study by Grossman et al. (2017), which provided the first empirical in-vivo evidence of tTIS effects in rodents, subsequent research has primarily established its efficacy through theoretical computer models (Rampersad et al. 2019; Esmaeilpour et al. 2021; Mirzakhalili et al. 2020; Negahbani et al. 2018). Only recently have additional studies added to the body of in-vivo research, demonstrating the effects of tTIS in rodents (Sunshine et al. 2021; Carmona-Barrón et al. 2023; Liu et al. 2023). However, research on the use of tTIS in humans remains limited. In this dissertation, I present novel and promising findings on the feasibility and efficacy of tTIS in human subjects.

The primary aim of Study 1 (Thiele et al. 2021) was to obtain a direct and easily measurable effect of tTIS, specifically by inducing phosphenes. Phosphenes are visual sensations of light that occur without actual light entering the eye and can be caused by pressure, neurological conditions, or electrical stimulation (Turi et al. 2013). Due to the high sensitivity of retinal cells to electrostimulation, phosphenes can serve as straightforward indicators of tES efficacy (Lindenblatt and Silny 2002). Therefore, the objective of this study was to demonstrate that amplitude-modulated waveforms could induce phosphenes, providing evidence of neuronal stimulation efficacy through activation of retinal neurons. Various stimulation frequencies were applied to the participants' retinas, and phosphene thresholds were measured. The hypothesis was that the amplitude-modulated signal would induce phosphenes, indicating its effectiveness in neuronal stimulation. Although we did not observe

phosphene induction via this waveform, the study provided valuable insights. Expanding these findings of amplitude-modulated waveforms to tTIS, the results suggest that tTIS may not activate retinal cells directly. However, this does not imply that tTIS lacks overall stimulation effects. Similar to other transcranial electrostimulation methods, such as tACS, the primary targeted mechanism of tTIS is neuronal *modulation*, not *activation*. With this understanding, neuronal modulation presented itself as the next promising avenue to establish a proof-of-concept for tTIS in humans.

Building on the findings from Study 1, Study 2 (Thiele et al. 2024a) aimed to adapt a well-established tACS paradigm to tTIS, specifically focusing on inducing parieto-occipital alpha entrainment during a mental rotation task. The objective was to assess the efficacy of tTIS in modulating alpha activity and to demonstrate neuronal modulation in humans. While entrainment effects are well-documented in tACS literature (Zaehle et al. 2010; Kasten and Herrmann 2017), no study had yet demonstrated them with tTIS in humans. Given that both tACS and tTIS are based on low-frequency stimulation signals, their entrainment effects should be comparable. This study compared the effects of tTIS, tACS, and a sham control on participants performing a mental rotation task. We hypothesized that both tTIS and tACS would increase parieto-occipital alpha activity, thereby enhancing task performance. Study 2 successfully demonstrated an outlasting electrophysiological effect of tTIS in the form of an increase in event-related desynchronization (ERD) in the alpha range during the mental rotation task. This increase in ERD suggests enhanced neural engagement or altered oscillatory activity associated with the task, providing new evidence for the neuromodulatory effect of tTIS in humans and extending previous tACS findings to tTIS. However, contrary to our hypothesis, we did not observe a significant effect of stimulation on mental rotation performance or resting alpha activity. The lack of behavioral improvement may be due to a ceiling effect, as participants achieved near-perfect accuracy, leaving little room for enhancement. Furthermore, no stimulation effect was observed on alpha activity during

rest periods, suggesting that tTIS stimulation effects are more apparent when an active endogenous neuronal network is engaged in task execution.

The aim of Study 3 (Thiele et al. 2024b) was to broaden the scope of tTIS by investigating the upper limits of feasible stimulation intensities for human applications. Traditionally, human stimulation studies use a maximum of 2 mA to avoid cutaneous adverse effects such as skin tingling or burning sensations (Khadka and Bikson 2022). However, tTIS offers the advantage that its high-frequency currents are less perceptible due to the intrinsic low-pass filtering of somatosensory receptors (Hutcheon and Yarom 2000), allowing for increased stimulation intensities without compromising participant comfort. In addition, recent studies have suggested that tTIS may require higher stimulation intensities than tACS to achieve comparable effects (Esmaeilpour et al. 2021; Negahbani et al. 2018). Therefore, we hypothesized that tTIS could be safely applied at intensities up to 4 mA without causing uncomfortable adverse effects. The study demonstrated that tTIS can be applied at higher intensities than conventional tACS, reaching up to 4 mA. Additionally, it showed that the use of a topical anesthetic can facilitate the application of higher intensities by reducing cutaneous sensations. This finding paves the way for future human tTIS research, which can benefit from increased stimulation intensity and, consequently, enhanced stimulation efficacy.

To summarize, this dissertation provides proof-of-concept for tTIS in humans by exploring and demonstrating its impact on various neuropsychological phenomena. These findings lay the groundwork for establishing tTIS as a viable method for human research and treatment, marking a significant step forward in the field.

2.2. Current and Future Prospects of tTIS

So far, we have primarily been in the proof-of-concept phase of tTIS. This is a necessary step to evaluate the method and analyze its feasibility and efficacy. Consequently, tTIS has not yet been widely

applied to concepts with real-world benefits. Instead, technical and foundational studies are the norm, focusing on understanding the mechanisms as well as the dos and don'ts of tTIS. However, as we move beyond this phase, we will be able to fully realize and reap the true benefits of tTIS.

From a neuroscientific perspective, tTIS offers the possibility to non-invasively modulate brain activity in deeper brain areas that current methods of transcranial electrostimulation, such as tACS, cannot effectively stimulate. Generally speaking, for neuroscientists, the biggest advantage of tES methods has always been the possibility to non-invasively achieve changes in brain activity within a specific area. Based on resulting changes in behavior or electrophysiology, this allows the user to draw not only correlative but, more importantly, causal inferences about function and role of the stimulated area (Paulus et al. 2016; Fertonani and Miniussi 2017; Beliaeva et al. 2021). For instance, it can be easily demonstrated that the occipital area is causally linked to visual perception, as altering activity within this region leads to changes in visual perception (Gonzalez-Perez et al. 2019; Antal et al. 2011).

With tTIS, this capability extends to other areas as well. Among the few human studies on tTIS available today, Violante et al. (2023) demonstrated the ability of tTIS to stimulate the human hippocampus. In their study, they first used computer models and human cadaver measurements to verify the steerability of the stimulation locus to the hippocampus while still achieving sufficient electric field strengths of approximately 0.3 V/m. They then performed an MRI study with participants, demonstrating that tTIS modulates hippocampal activity and enhances memory performance during a face-name pairing task. Another study by Zhang et al. (2022a) targeted frontoparietal areas to improve working memory performance, Ma et al. (2021) stimulated the primary motor cortex (M1) to enhance motor learning and Zheng et al. (2024) achieved an improvement in vertical jumping height, also after M1 stimulation. However, many questions about tTIS remain. For example, Conta et al. (2022) and Iszak et al. (2023) unsuccessfully attempted to generate entrainment of resting alpha activity in parieto-occipital areas after tTIS. This finding aligns with my Study 2, where I was unable to observe an effect

on resting alpha power. Instead, I demonstrated that tTIS requires an active network, engaged in task performance, for stimulation effects to manifest.

From a neurotherapeutic perspective, tTIS could prove to be an even more valuable tool. As previously described, current deep-brain stimulation approaches could potentially be facilitated or even replaced by tTIS. Among the most prominent and promising applications is the treatment of Parkinson's disease (Grossman et al. 2018). Currently, an invasive electrode must be implanted intracranially to modulate the motor network activity and treat Parkinson's symptoms. Future studies should evaluate whether tTIS can achieve comparable effects. While tTIS may not fully replace DBS for advanced Parkinson's patients, it could serve as an initial treatment for mild cases or early stages of the disease, potentially delaying the need for DBS surgery or controlling symptoms until a DBS operation becomes necessary.

Another promising avenue for tTIS is in the treatment of epileptiform seizures. As demonstrated in mice, tTIS can reduce epileptiform discharges, suggesting its potential to modulate epileptic foci and reduce symptoms (Missey et al. 2021; Acerbo et al. 2022). Other studies have begun to apply the principle of temporal interference to areas beyond the brain. For example, Sunshine et al. (2021) used temporal interference stimulation to rescue rodents whose respiratory flow was stopped with fentanyl. By targeting the diaphragm, the stimulation induced muscle activity, allowing the rodents to artificially resume breathing. In a first therapeutic study in humans, temporal interference stimulation was used to treat obstructive sleep apnea (Missey et al. 2023). In this study, the hypoglossal nerve was stimulated, stabilizing the position of the tongue in a protruded state. This prevented the collapse of the airway, thereby allowing for free airflow.

As of today, we have only scratched the surface of tTIS research, and there is tremendous potential to explore and harness its benefits. The possibilities for future applications of tTIS are vast, and the field holds great promise for advancing our understanding and treatment of various neurological conditions.

2.3. New Developments for tTIS

A novel approach to performing tTIS has recently been explored, known as transcranial pulse-width modulated temporal interference stimulation (PWM-TI) (see **Figure 5**). This new variation involves applying two high-frequency square waves instead of sine waves. Similar to tTIS, these square-wave signals share a frequency offset Δf (e.g., $\Delta f = f_1 - f_2$ = 1020 Hz – 1000 Hz = 20 Hz), maintaining the principles of destructive and constructive interference. However, due to the square-wave nature of the signals, this does not generate an amplitude-modulated waveform. Instead, the width of the square wave is modulated. Essentially, this means that the duration at which the signal stays at full intensity before shifting downwards is periodically increased and decreased in the Δf rhythm.

While it is not yet fully understood if or how PWM-TI differs from sinusoidal tTIS, initial results look promising. Missey et al. (2021) successfully used this method to induce seizure-like events (SLEs) in the mouse hippocampus, evidenced by behavioral seizure symptoms such as freezing, head nodding, or forelimb clonus. These findings are substantiated by Acerbo et al. (2022), who performed PWM-TI stimulation in a mouse model of epilepsy, reducing the number of epileptiform discharges in the hippocampus. It is important to note that while Missey et al. (2021) aimed to induce epileptic activity via PWM-TI, Acerbo et al. (2022) took the opposite approach, attempting to reduce epileptic activity in an already epileptic mouse model. Furthermore, to demonstrate the successful propagation of the PWM-TI signal in larger brains, Acerbo et al. (2022) applied PWM-TI to a human cadaver. They were able to demonstrate a clear pulse-width modulated waveform in the targeted hippocampus, showcasing PWM-TI's capability to reach deeper target areas in larger (human) brains. As of today, there is only one other study utilizing PWM-TI, conducted by Luff et al. (2023). They used single-cell recordings in mice to demonstrate that a neuron's membrane potential can be successfully entrained to fluctuate in-phase with the applied stimulation signal, leading to modulation of the subthreshold amplitude. Additionally, using calcium imaging, they proved that network activity in the target area was also

successfully modulated. Moreover, they compared classical tTIS to PWM-TI and found an efficacy advantage with PWM-TI, which required 20% less stimulation intensity to induce oscillatory activity. Based on these findings, it would be worthwhile to explore PWM-TI and the underlying biophysics to further tap into this promising variation of the classical tTIS principle.

Another exciting new avenue for tTIS is its application in a theta-burst rhythm (see **Figure 6**), recently introduced by (Wessel et al. 2023). In this innovative variation, two high-frequency fields of 2 kHz are used, but five times per second, one of the fields briefly shifts to 2.1 kHz. This shift introduces a 100 Hz envelope in short bursts that mimic theta rhythm. Theta-burst stimulation has been shown to induce long-term potentiation (LTP)-like effects in neurons (Huang et al. 2005), and the theta-burst tTIS is hypothesized to increase neuronal activity through similar mechanisms. In their study, Wessel et al. applied theta-burst tTIS to the human striatum and observed increased neuronal activity in the putamen,

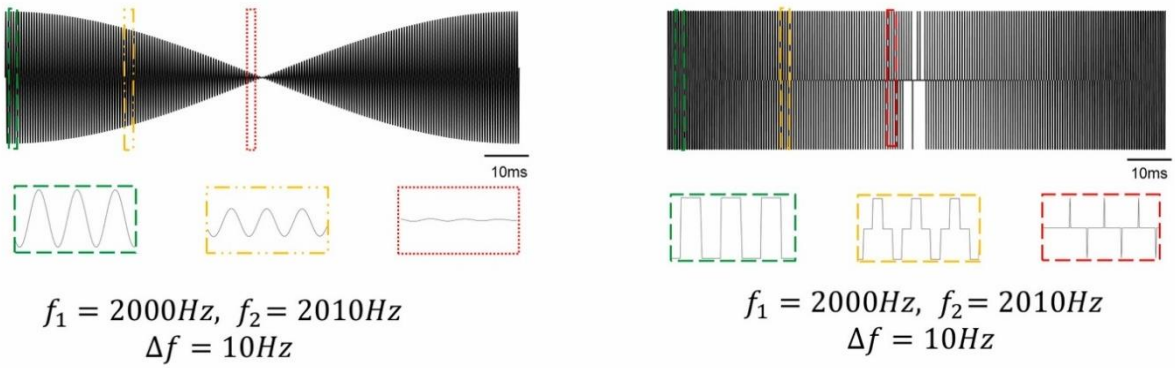

Figure 5 | TTIS vs. PWM-TI. **(Left)** An amplitude-modulated waveform generated by tTIS. This waveform results from the interference of two high-frequency sinusoidal signals, creating varying points of maximum constructive interference (green) where the polarities of both electric fields are perfectly aligned. This is followed by a phase where the signals shift out of sync, resulting in equal strength of positive and destructive interference (yellow). The state then shifts to where destructive interference outweighs constructive interference, with the fields nearly in anti-phase (red). Eventually, the fields reach a perfect anti-phase state (not marked, zero-point just right of the red-marked area), where they completely cancel each other out and the cycle begins anew. **(Right)** A pulse-width modulated signal generated by PWM-TI. This summed signal arises from the application of two high-frequency square waves. The synchronization shifts periodically in the Δf rhythm, transitioning from a perfectly in-phase state of both square waves (green), where the peak of the stimulation is at its full width, to a general decrease in peak width and the appearance of a lower secondary flat shelf (yellow), until a near-zero sum is achieved when the signals are in anti-phase (red). *Note*. Taken from Luff et al. (2023) and reprinted under Creative Commons CC-BY license.

as demonstrated by elevated blood-oxygenation-level-dependent (BOLD) signals. Additionally, they reported improved performance in a serialized finger tapping task among older adults. However, as of now, theta-burst tTIS remains a largely unexplored possibility, and further research is needed to evaluate its potential for human application.

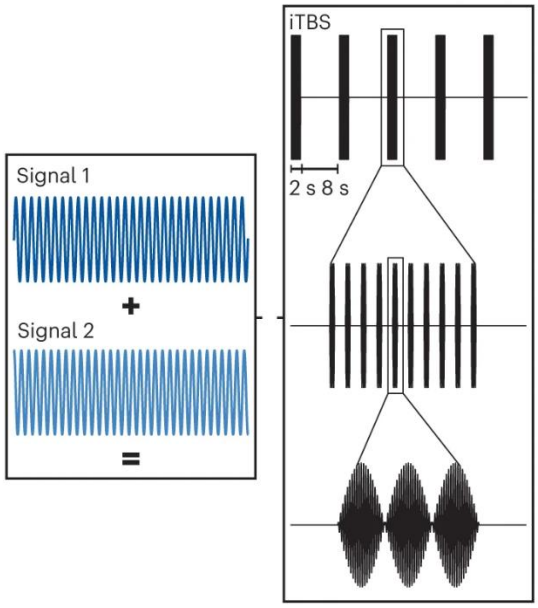

Figure 6 | Theta-Burst tTIS. Two sinusoidal high-frequency alternating currents are applied, with the key aspect that the frequency difference Δf (e.g., $\Delta f = f1 - f2$ = 1010 Hz - 1000 Hz = 10 Hz) is introduced only for short bursts. For the remainder of the duration, both alternating currents share the same frequency (e.g., $\Delta f = f1 - f2$ = 1000 Hz - 1000 Hz = 0 Hz). *Note*. Taken from Wessel et al. (2023) and reprinted under Creative Commons CC-BY license.

2.4. Limitations and Solutions

One of the key strengths of tTIS—its high focality and lack of off-target stimulation—can also present limitations. The stimulation locus is defined as the area with the greatest amplitude-modulated waveform, generated via the superposition of both electric fields at equal intensity (Grossman et al. 2017). This requires an electrode montage that precisely guides the stimulation locus to the target area. Additionally, since the locus of stimulation is determined by the current ratio between both applied electric fields, it introduces an additional degree of freedom and uncertainty. Moreover, the direction of the electric fields is crucial, as tTIS primarily works through axonal stimulation. This stimulation is

most effective when the electric field aligns with the orientation of the axon and significantly less so when perpendicular to it (Mirzakhalili et al. 2020; Esmaeilpour et al. 2021). Thus, it becomes essential to also consider the orientation of neurons in the target area.

As such, precise modeling of the electrode montage is essential before tTIS application to ensure optimal distribution of the electric fields. Studies have shown that the electric flow within the brain is significantly affected by neuroanatomical features such as the thickness of the subcutaneous fat layer, skull thickness, and the thickness of cerebrospinal fluid (for a review see Hunold et al. 2023). This implies that ideal stimulation will always necessitate neuroanatomical imaging to model individualized electric field distributions for each subject (Cassara et al. 2021; Lee et al. 2020; Stoupis and Samaras 2022; Conta et al. 2021; Bahn et al. 2023). Furthermore, current modeling methods can reliably provide only a forward solution for field distribution, meaning they simulate current density in the target area given a chosen electrode montage. Considering the infinite possibilities spanning the full spectrum of potential stimulation montages—such as electrode positions, electrode sizes, current ratios, number of electrodes, carrier frequencies, or envelope frequencies—the search for the optimal stimulation setup is akin to finding a needle in a haystack.

What is needed are models capable of providing an inverse solution to this problem. This means that models should be able to determine the optimal stimulation setup for a given target area. Currently, preliminary models offer approximate solutions using a discrete set of parameters, such as the 64 possible electrode positions of the 10-10 system (Cassara et al. 2021; Lee et al. 2020; Stoupis and Samaras 2022; Bahn et al. 2023). However, these models are still under development and not yet readily available.

To sum up, optimizing the efficacy of tTIS requires the use of individualized neuroanatomical modeling of electric fields to determine the best electrode setups. This approach ensures that the target

area receives sufficiently high electric currents for effective stimulation. While this method is optional and costly, it guarantees the highest quality of tTIS application, leveraging its full potential.

2.5. Conclusion

To conclude, tTIS holds significant promise as a valuable addition to neuroscience and clinical treatment. This dissertation provides a proof-of-concept for tTIS in humans by exploring and demonstrating its impact on various neuropsychological phenomena. These findings lay the groundwork for establishing tTIS as a viable method for both human research and therapeutic interventions, marking a significant advancement in the field. With numerous potential applications ranging from fundamental neuroscience research to clinical therapies, tTIS offers a versatile tool for advancing our understanding of the brain and modulating its activity. Looking ahead, further exploration of these applications is anticipated, which will likely enhance our comprehension of neural mechanisms and improve the quality of life for patients through improved treatments.

3. Publication bibliography

Abrams, R. (1989): Electroconvulsive therapy. In *Current opinion in psychiatry* 2 (1), p. 63.

Acerbo, Emma; Jegou, Aude; Luff, Charlotte; Dzialecka, Patrycja; Botzanowski, Boris; Missey, Florian et al. (2022): Focal non-invasive deep-brain stimulation with temporal interference for the suppression of epileptic biomarkers. In *Frontiers in neuroscience* 16, p. 945221. DOI: 10.3389/fnins.2022.945221.

Albarran, Tyler (2023): Optimization of Temporal Interference Stimulation for Invasive Neuromodulation of Motor Neurons Using an In-Silico Sciatic Nerve Model.

Albornoz-Cabello, Manuel; Pérez-Mármol, José Manuel; Barrios Quinta, Cristo Jesus; Matarán-Peñarrocha, Guillermo A.; Castro-Sánchez, Adelaida María; La Cruz Olivares, Blanca de (2019): Effect of adding interferential current stimulation to exercise on outcomes in primary care patients with chronic neck pain: a randomized controlled trial. In *Clinical Rehabilitation* 33 (9), pp. 1458–1467. DOI: 10.1177/0269215519844554.

Alexander, Morgan L.; Alagapan, Sankaraleengam; Lugo, Courtney E.; Mellin, Juliann M.; Lustenberger, Caroline; Rubinow, David R.; Fröhlich, Flavio (2019): Double-blind, randomized pilot clinical trial targeting alpha oscillations with transcranial alternating current stimulation (tACS) for the treatment of major depressive disorder (MDD). In *Translational psychiatry* 9 (1), pp. 1–12.

Antal, Andrea; Boros, Klára; Poreisz, Csaba; Chaieb, Leila; Terney, Daniella; Paulus, Walter (2008): Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. In *Brain stimulation* 1 (2), pp. 97–105.

Antal, Andrea; Kincses, Tamás Z.; Nitsche, Michael A.; Paulus, Walter (2003): Manipulation of phosphene thresholds by transcranial direct current stimulation in man. In *Experimental brain research* 150 (3), pp. 375–378. DOI: 10.1007/s00221-003-1459-8.

Antal, Andrea; Paulus, Walter (2013): Transcranial alternating current stimulation (tACS). In *Front. Hum. Neurosci.* 7, p. 317. DOI: 10.3389/fnhum.2013.00317.

Antal, Andrea; Paulus, Walter; Nitsche, Michael A. (2011): Electrical stimulation and visual network plasticity. In *Restorative neurology and neuroscience* 29 (6), pp. 365–374. DOI: 10.3233/RNN-2011-0609.

Ashkan, Keyoumars; Rogers, Priya; Bergman, Hagai; Ughratdar, Ismail (2017): Insights into the mechanisms of deep brain stimulation. In *Nat Rev Neurol* 13 (9), pp. 548–554. DOI: 10.1038/nrneurol.2017.105.

Bahn, Sangkyu; Lee, Chany; Kang, Bo-Yeong (2023): A computational study on the optimization of transcranial temporal interfering stimulation with high-definition electrodes using unsupervised neural networks. In *Human Brain Mapping* 44 (5), pp. 1829–1845. DOI: 10.1002/hbm.26181.

Beatti, Abulkhair; Rayner, Anton; Chipchase, Lucy; Souvlis, Tina (2011): Penetration and spread of interferential current in cutaneous, subcutaneous and muscle tissues. In *Physiotherapy* 97 (4), pp. 319–326. DOI: 10.1016/j.physio.2011.01.008.

Beliaeva, Valeriia; Savvateev, Iurii; Zerbi, Valerio; Polania, Rafael (2021): Toward integrative approaches to study the causal role of neural oscillations via transcranial electrical stimulation. In *Nat Commun* 12 (1), p. 2243. DOI: 10.1038/s41467-021-22468-7.

Berger, Hans (1929): Über das elektroenkephalogramm des menschen. In *Archiv für psychiatrie und nervenkrankheiten* 87 (1), pp. 527–570.

Bieler, Lara Sabrina: Effects of transcranial alternating current stimulation (tACS) on different aspects of general motor skill and implicit motor sequence learning in healthy young and older individuals depending on stimulation frequency / Lara Sabrina Bieler. Karl-Franzens-Universität Graz. Available online at https://unipub.uni-graz.at/obvugrhs/2801172.

Bikson, Marom; Grossman, Pnina; Thomas, Chris; Zannou, Adantchede Louis; Jiang, Jimmy; Adnan, Tatheer et al. (2016): Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016. In *Brain stimulation* 9 (5), pp. 641–661. DOI: 10.1016/j.brs.2016.06.004.

Bland, Nicholas S.; Sale, Martin V. (2019): Current challenges: the ups and downs of tACS. In *Exp Brain Res* 237 (12), pp. 3071–3088. DOI: 10.1007/s00221-019-05666-0.

Buzsáki, György; Anastassiou, Costas A.; Koch, Christof (2012): The origin of extracellular fields and currents--EEG, ECoG, LFP and spikes. In *Nat Rev Neurosci* 13 (6), pp. 407–420. DOI: 10.1038/nrn3241.

Cao, Jiaming; Doiron, Brent; Goswami, Chaitanya; Grover, Pulkit (2020): The Mechanics of Temporal Interference Stimulation. In *bioRxiv*, 2020.04.23.051870. DOI: 10.1101/2020.04.23.051870.

Carmona-Barrón, Venezia G.; Del Fernández Campo, Inés S.; Delgado-García, José M.; La Fuente, Antonio J. de; Lopez, Ignacio Plaza; Merchán, Miguel A. (2023): Comparing the effects of transcranial alternating current and temporal interference (tTIS) electric stimulation through whole-brain mapping of c-Fos immunoreactivity. In *Front. Neuroanat.* 17, p. 1128193. DOI: 10.3389/fnana.2023.1128193.

Cassara, Antonino M.; Neufeld, Esra; Capstick, Myles; Grossman, Nir; Kuster, Niels (2021): 'Intelligent'Current Source And Computational Modeling for Personalized Temporal Interference Brain Stimulation From DC–100 kHz.

Cassarà, Antonino M.; Newton, Taylor H.; Zhuang, Katie; Regel, Sabine J.; Achermann, Peter; Kuster, Niels; Neufeld, Esra (2022): Safety Recommendations for Temporal Interference Stimulation in the Brain. In *bioRxiv*, 2022.12.15.520077. DOI: 10.1101/2022.12.15.520077.

Conta, Jill von; Kasten, Florian H.; Ćurčić-Blake, Branislava; Aleman, André; Thielscher, Axel; Herrmann, Christoph S. (2021): Interindividual variability of electric fields during transcranial temporal interference stimulation (tTIS). In *Scientific reports* 11 (1), p. 20357. DOI: 10.1038/s41598-021-99749-0.

Conta, Jill von; Kasten, Florian H.; Schellhorn, Klaus; Ćurčić-Blake, Branislava; Aleman, André; Herrmann, Christoph S. (2022): Benchmarking the effects of transcranial temporal interference stimulation (tTIS) in humans. In *Cortex* 154, pp. 299–310. DOI: 10.1016/j.cortex.2022.05.017.

Elyamany, Osama; Leicht, Gregor; Herrmann, Christoph S.; Mulert, Christoph (2021): Transcranial alternating current stimulation (tACS): from basic mechanisms towards first applications in psychiatry. In *Eur Arch Psychiatry Clin Neurosci* 271 (1), pp. 135–156. DOI: 10.1007/s00406-020-01209-9.

Esmaeilpour, Zeinab; Kronberg, Greg; Reato, Davide; Parra, Lucas C.; Bikson, Marom (2021): Temporal interference stimulation targets deep brain regions by modulating neural oscillations. In *Brain stimulation* 14 (1), 55-65. DOI: 10.1016/j.brs.2020.11.007.

Espinoza, Randall T.; Kellner, Charles H. (2022): Electroconvulsive Therapy. In *The New England journal of medicine* 386 (7), pp. 667–672. DOI: 10.1056/NEJMra2034954.

Fertonani, Anna; Ferrari, Clarissa; Miniussi, Carlo (2015): What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. In *Clinical Neurophysiology* 126 (11), pp. 2181–2188. DOI: 10.1016/j.clinph.2015.03.015.

Fertonani, Anna; Miniussi, Carlo (2017): Transcranial electrical stimulation: what we know and do not know about mechanisms. In *The Neuroscientist* 23 (2), pp. 109–123.

Fowler, Michael (1997): Historical beginnings of theories of electricity and magnetism. In *Recuperado el* 23 (5), p. 2012.

Francis, J.; Dingley, J. (2015): Electroanaesthesia--from torpedo fish to TENS. In *Anaesthesia* 70 (1), pp. 93–103. DOI: 10.1111/anae.12887.

Goats, G. C. (1990): Interferential current therapy. In *British journal of sports medicine* 24 (2), pp. 87–92. DOI: 10.1136/bjsm.24.2.87.

Gomez-Tames, Jose; Asai, Akihiro; Hirata, Akimasa (2021): Multiscale Computational Model Reveals Nerve Response in a Mouse Model for Temporal Interference Brain Stimulation. In *Frontiers in neuroscience* 15, p. 684465. DOI: 10.3389/fnins.2021.684465.

Gonzalez-Perez, Montserrat; Wakui, Elley; Thoma, Volker; Nitsche, Michael A.; Rivolta, Davide (2019): Transcranial alternating current stimulation (tACS) at 40 Hz enhances face and object perception. In *Neuropsychologia* 135, p. 107237. DOI: 10.1016/j.neuropsychologia.2019.107237.

Grossman, Nir; Bono, David; Dedic, Nina; Kodandaramaiah, Suhasa B.; Rudenko, Andrii; Suk, Ho-Jun et al. (2017): Noninvasive deep brain stimulation via temporally interfering electric fields. In *Cell* 169 (6), 1029-1041. e16. DOI: 10.1016/j.cell.2017.05.024.

Grossman, Nir; Okun, Michael S.; Boyden, Edward S. (2018): Translating Temporal Interference Brain Stimulation to Treat Neurological and Psychiatric Conditions. In *JAMA neurology* 75 (11), pp. 1307–1308. DOI: 10.1001/jamaneurol.2018.2760.

Hankinson, R. J. (1998): Cause and Explanation in Ancient Greek Thought. Oxford, Ann Arbor, Michigan: Oxford University Press; ProQuest.

Herrmann, Christoph S.; Rach, Stefan; Neuling, Toralf; Strüber, Daniel (2013): Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. In *Front. Hum. Neurosci.* 7, p. 279. DOI: 10.3389/fnhum.2013.00279.

Howell, Bryan; McIntyre, Cameron C. (2021): Feasibility of Interferential and Pulsed Transcranial Electrical Stimulation for Neuromodulation at the Human Scale. In *Neuromodulation* 24 (5), pp. 843–853. DOI: 10.1111/ner.13137.

Hoy, Kate E.; Bailey, Neil; Arnold, Sara; Windsor, Kirstyn; John, Joshua; Daskalakis, Zafiris J.; Fitzgerald, Paul B. (2015): The effect of γ-tACS on working memory performance in healthy controls. In *Brain and Cognition* 101, pp. 51–56. DOI: 10.1016/j.bandc.2015.11.002.

Huang, Wei A.; Stitt, Iain M.; Negahbani, Ehsan; Passey, D. J.; Ahn, Sangtae; Davey, Marshall et al. (2021): Transcranial alternating current stimulation entrains alpha oscillations by preferential

phase synchronization of fast-spiking cortical neurons to stimulation waveform. In *Nat Commun* 12 (1), p. 3151. DOI: 10.1038/s41467-021-23021-2.

Huang, Ying-Zu; Edwards, Mark J.; Rounis, Elisabeth; Bhatia, Kailash P.; Rothwell, John C. (2005): Theta burst stimulation of the human motor cortex. In *Neuron* 45 (2), pp. 201–206. DOI: 10.1016/j.neuron.2004.12.033.

Huang, Yu; Datta, Abhishek; Bikson, Marom; Parra, Lucas C. (2019): Realistic volumetric-approach to simulate transcranial electric stimulation-ROAST-a fully automated open-source pipeline. In *J. Neural Eng.* 16 (5), p. 56006. DOI: 10.1088/1741-2552/ab208d.

Hunold, Alexander; Haueisen, Jens; Nees, Frauke; Moliadze, Vera (2023): Review of individualized current flow modeling studies for transcranial electrical stimulation. In *Journal of Neuroscience Research* 101 (4), pp. 405–423. DOI: 10.1002/jnr.25154.

Hutcheon, B.; Yarom, Y. (2000): Resonance, oscillation and the intrinsic frequency preferences of neurons. In *Trends in neurosciences* 23 (5), pp. 216–222. DOI: 10.1016/s0166-2236(00)01547-2.

Iszak, Krisztián; Gronemann, Simon Mathies; Meyer, Stefanie; Hunold, Alexander; Zschüntzsch, Jana; Bähr, Mathias et al. (2023): Why Temporal Inference Stimulation May Fail in the Human Brain: A Pilot Research Study. In *Biomedicines* 11 (7), p. 1813. DOI: 10.3390/biomedicines11071813.

Jackson, Mark P.; Truong, Dennis; Brownlow, Milene L.; Wagner, Jessica A.; McKinley, R. Andy; Bikson, Marom; Jankord, Ryan (2017): Safety parameter considerations of anodal transcranial direct current stimulation in rats. In *Brain, Behavior, and Immunity* 64, pp. 152–161.

Jacobs, Joshua; Kahana, Michael J.; Ekstrom, Arne D.; Fried, Itzhak (2007): Brain oscillations control timing of single-neuron activity in humans. In *J. Neurosci.* 27 (14), pp. 3839–3844. DOI: 10.1523/JNEUROSCI.4636-06.2007.

Karimi, Fariba; Attarpour, Ahmadreza; Amirfattahi, Rassoul; Nezhad, Abolghasem Zeidaabadi (2019): Computational analysis of non-invasive deep brain stimulation based on interfering electric fields. In *Physics in medicine and biology* 64 (23), p. 235010. DOI: 10.1088/1361-6560/ab5229.

Kasten, Florian H.; Herrmann, Christoph S. (2017): Transcranial Alternating Current Stimulation (tACS) Enhances Mental Rotation Performance during and after Stimulation. In *Front. Hum. Neurosci.* 11, p. 2. DOI: 10.3389/fnhum.2017.00002.

Khadka, Niranjan; Bikson, Marom (2022): Noninvasive Electrical Brain Stimulation of the Central Nervous System. In *Handbook of Neuroengineering*, pp. 1–33.

Lasbareilles, Camille; Pogosyan, Alek; Mancini, Valentina; Tan, Huiling; Stagg, Charlotte (2023): The functional role of theta-gamma oscillations in healthy human motor learning using theta-gamma phase amplitude coupling tACS. In *1935-861X* 16 (1), pp. 261–262. DOI: 10.1016/j.brs.2023.01.432.

Lee, Sangjun; Lee, Chany; Park, Jimin; Im, Chang-Hwan (2020): Individually customized transcranial temporal interference stimulation for focused modulation of deep brain structures: a simulation study with different head models. In *Sci Rep* 10 (1), p. 11730. DOI: 10.1038/s41598-020-68660-5.

Liebetanz, David; Koch, Reinhard; Mayenfels, Susanne; König, Fatima; Paulus, Walter; Nitsche, Michael A. (2009): Safety limits of cathodal transcranial direct current stimulation in rats. In

Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology 120 (6), pp. 1161–1167. DOI: 10.1016/j.clinph.2009.01.022.

Limousin, Patricia; Foltynie, Tom (2019): Long-term outcomes of deep brain stimulation in Parkinson disease. In *Nat Rev Neurol* 15 (4), pp. 234–242. DOI: 10.1038/s41582-019-0145-9.

Lindenblatt, G.; Silny, J. (2002): Electrical phosphenes: on the influence of conductivity inhomogeneities and small-scale structures of the orbita on the current density threshold of excitation. In *Medical & biological engineering & computing* 40 (3), pp. 354–359.

Liu, Ruobing; Zhu, Guanyu; Wu, Zhengping; Gan, Yifei; Zhang, Jianguo; Liu, Jiali; Wang, Liang (2024): Temporal interference stimulation targets deep primate brain. In *Neuroimage* 291, p. 120581. DOI: 10.1016/j.neuroimage.2024.120581.

Liu, Xiaodong; Qi, Shuo; Hou, Lijuan; Liu, Yu; Wang, Xiaohui (2023): Noninvasive deep brain stimulation via temporal interference electric fields enhanced motor performance of mice and its neuroplasticity mechanisms. DOI: 10.21203/rs.3.rs-2993391/v1.

Lloyd, Geoffrey (1975): Alcmaeon and the early history of dissection. In *Sudhoffs Archiv*, pp. 113–147.

Lozano, Andres M.; Lipsman, Nir; Bergman, Hagai; Brown, Peter; Chabardes, Stephan; Chang, Jin Woo et al. (2019): Deep brain stimulation: current challenges and future directions. In *Nat Rev Neurol* 15 (3), pp. 148–160. DOI: 10.1038/s41582-018-0128-2.

Luff, Charlotte E.; Dzialecka, Patrycja; Acerbo, Emma; Williamson, Adam; Grossman, Nir (2023): Pulse-width modulated temporal interference (PWM-TI) brain stimulation. In *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation* 17 (1), pp. 92–103. DOI: 10.1016/j.brs.2023.12.010.

Ma, Ru; Xia, Xinzhao; Zhang, Wei; Lu, Zhuo; Wu, Qianying; Cui, Jiangtian et al. (2021): High Gamma and Beta Temporal Interference Stimulation in the Human Motor Cortex Improves Motor Functions. In *Frontiers in neuroscience* 15. DOI: 10.3389/fnins.2021.800436.

Marchesotti, Silvia; Nicolle, Johanna; Merlet, Isabelle; Arnal, Luc H.; Donoghue, John P.; Giraud, Anne-Lise (2020): Selective enhancement of low-gamma activity by tACS improves phonemic processing and reading accuracy in dyslexia. In *PLoS biology* 18 (9), e3000833. DOI: 10.1371/journal.pbio.3000833.

Matsumoto, Hideyuki; Ugawa, Yoshikazu (2017): Adverse events of tDCS and tACS: a review. In *Clinical Neurophysiology Practice* 2, pp. 19–25.

Meng, Hao; Houston, Michael; Zhang, Yingchun; Li, Sheng (2024): Exploring the Prospects of Transcranial Electrical Stimulation (tES) as a Therapeutic Intervention for Post-Stroke Motor Recovery: A Narrative Review. In *Brain Sciences* 14 (4), p. 322. DOI: 10.3390/brainsci14040322.

Mirzakhalili, Ehsan; Barra, Beatrice; Capogrosso, Marco; Lempka, Scott F. (2020): Biophysics of Temporal Interference Stimulation. In *Cell Systems* 11 (6), 557-572.e5. DOI: 10.1016/j.cels.2020.10.004.

Missey, Florian; Ejneby, Malin Silverå; Ngom, Ibrahima; Donahue, Mary J.; Trajlinek, Jan; Acerbo, Emma et al. (2023): Obstructive sleep apnea improves with non-invasive hypoglossal nerve stimulation using temporal interference. In *Bioelectronic medicine* 9 (1), p. 18. DOI: 10.1186/s42234-023-00120-7.

Missey, Florian; Rusina, Evgeniia; Acerbo, Emma; Botzanowski, Boris; Trébuchon, Agnès; Bartolomei, Fabrice et al. (2021): Orientation of temporal interference for non-invasive deep brain stimulation in epilepsy. In *Front. Neurosci.* 15, p. 633988.

Miyaguchi, Shota; Otsuru, Naofumi; Kojima, Sho; Yokota, Hirotake; Saito, Kei; Inukai, Yasuto; Onishi, Hideaki (2019): Gamma tACS over M1 and cerebellar hemisphere improves motor performance in a phase-specific manner. In *Neuroscience Letters* 694, pp. 64–68. DOI: 10.1016/j.neulet.2018.11.015.

Naros, Georgios; Gharabaghi, Alireza (2017): Physiological and behavioral effects of β-tACS on brain self-regulation in chronic stroke. In *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation* 10 (2), pp. 251–259. DOI: 10.1016/j.brs.2016.11.003.

Negahbani, Ehsan; Kasten, Florian H.; Herrmann, Christoph S.; Fröhlich, Flavio (2018): Targeting alpha-band oscillations in a cortical model with amplitude-modulated high-frequency transcranial electric stimulation. In *Neuroimage* 173, pp. 3–12. DOI: 10.1016/j.neuroimage.2018.02.005.

Nitsche, M. A.; Niehaus, L.; Hoffmann, K. T.; Hengst, S.; Liebetanz, D.; Paulus, W.; Meyer, B-U (2004): MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. In *Clinical Neurophysiology* 115 (10), pp. 2419–2423. DOI: 10.1016/j.clinph.2004.05.001.

Nitsche, M. A.; Paulus, W. (2000): Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. In *The Journal of physiology* 527 Pt 3, pp. 633–639. DOI: 10.1111/j.1469-7793.2000.t01-1-00633.x.

Nitsche, M. A.; Paulus, W. (2001): Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. In *Neurology* 57 (10), pp. 1899–1901. DOI: 10.1212/wnl.57.10.1899.

Nitsche, Michael A.; Schauenburg, Astrid; Lang, Nicolas; Liebetanz, David; Exner, Cornelia; Paulus, Walter; Tergau, Frithjof (2003): Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. In *Journal of cognitive neuroscience* 15 (4), pp. 619–626.

Pahor, Anja; Jaušovec, Norbert (2018): The effects of theta and gamma tACS on working memory and electrophysiology. In *Front. Hum. Neurosci.* 11, p. 651. DOI: 10.3389/fnhum.2017.00651.

Paulus, Walter; Nitsche, Michael A.; Antal, Andrea (2016): Application of transcranial electric stimulation (tDCS, tACS, tRNS): From motor-evoked potentials towards modulation of behaviour. In *European Psychologist* 21 (1), p. 4.

Piao, Yi; Ma, Ru; Weng, Yaohao; Fan, Chuan; Xia, Xinzhao; Zhang, Wei et al. (2022): Safety Evaluation of Employing Temporal Interference Transcranial Alternating Current Stimulation in Human Studies. In *Brain Sciences* 12 (9). DOI: 10.3390/brainsci12091194.

Pikovsky, Arkady; Rosenblum, Michael; Kurths, Jürgen (2001): Synchronization: A universal concept in nonlinear sciences.

Puonti, Oula; van Leemput, Koen; Saturnino, Guilherme B.; Siebner, Hartwig R.; Madsen, Kristoffer H.; Thielscher, Axel (2020): Accurate and robust whole-head segmentation from magnetic resonance images for individualized head modeling. In *Neuroimage* 219, p. 117044. DOI: 10.1016/j.neuroimage.2020.117044.

Ramirez-Zamora, Adolfo; Ostrem, Jill L. (2018): Globus Pallidus Interna or Subthalamic Nucleus Deep Brain Stimulation for Parkinson Disease: A Review. In *JAMA Neurol* 75 (3), pp. 367–372. DOI: 10.1001/jamaneurol.2017.4321.

Rampersad, Sumientra; Roig-Solvas, Biel; Yarossi, Mathew; Kulkarni, Praveen P.; Santarnecchi, Emiliano; Dorval, Alan D.; Brooks, Dana H. (2019): Prospects for transcranial temporal interference stimulation in humans: A computational study. In *Neuroimage* 202, p. 116124. DOI: 10.1016/j.neuroimage.2019.116124.

Read, John; Bentall, Richard (2010): The effectiveness of electroconvulsive therapy: a literature review. In *Epidemiologia e psichiatria sociale* 19 (4), pp. 333–347. DOI: 10.1017/s1121189x00000671.

Reato, Davide; Rahman, Asif; Bikson, Marom; Parra, Lucas C. (2010): Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. In *J. Neurosci.* 30 (45), pp. 15067–15079. DOI: 10.1523/JNEUROSCI.2059-10.2010.

Reed, Thomas; Cohen Kadosh, Roi (2018): Transcranial electrical stimulation (tES) mechanisms and its effects on cortical excitability and connectivity. In *J Inherit Metab Dis* 41 (6), pp. 1123–1130. DOI: 10.1007/s10545-018-0181-4.

Riddle, Justin; Rubinow, David R.; Frohlich, Flavio (2020): A case study of weekly tACS for the treatment of major depressive disorder. In *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation* 13 (3), pp. 576–577. DOI: 10.1016/j.brs.2019.12.016.

Röhner, Franziska; Breitling, Carolin; Rufener, Katharina S.; Heinze, Hans-Jochen; Hinrichs, Hermann; Krauel, Kerstin; Sweeney-Reed, Catherine M. (2018): Modulation of Working Memory Using Transcranial Electrical Stimulation: A Direct Comparison Between TACS and TDCS. In *Frontiers in neuroscience* 12, p. 761. DOI: 10.3389/fnins.2018.00761.

Rufener, Katharina S.; Zaehle, Tino (2021): Dysfunctional auditory gamma oscillations in developmental dyslexia: A potential target for a tACS-based intervention. In *Progress in Brain Research* 264, pp. 211–232. DOI: 10.1016/bs.pbr.2021.01.016.

Rufener, Katharina S.; Zaehle, Tino; Oechslin, Mathias S.; Meyer, Martin (2016): 40Hz-Transcranial alternating current stimulation (tACS) selectively modulates speech perception. In *International Journal of Psychophysiology* 101, pp. 18–24. DOI: 10.1016/j.ijpsycho.2016.01.002.

Ruhnau, Philipp; Neuling, Toralf; Fuscá, Marco; Herrmann, Christoph S.; Demarchi, Gianpaolo; Weisz, Nathan (2016): Eyes wide shut: Transcranial alternating current stimulation drives alpha rhythm in a state dependent manner. In *Scientific reports* 6, p. 27138. DOI: 10.1038/srep27138.

Schuhmann, Teresa; Kemmerer, Selma K.; Duecker, Felix; Graaf, Tom A. de; Oever, Sanne ten; Weerd, Peter de; Sack, Alexander T. (2019): Left parietal tACS at alpha frequency induces a shift of visuospatial attention. In *PLOS ONE* 14 (11), e0217729.

Song, Sixian; Zhang, Jiajia; Tian, Yi; Wang, Liping; Wei, Pengfei (2021): Temporal Interference Stimulation Regulates Eye Movements and Neural Activity in the Mice Superior Colliculus. In *43rd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)* 2021, pp. 6231–6234. DOI: 10.1109/embc46164.2021.9629968.

Stoupis, Dimitrios; Samaras, Theodoros (2022): Non-invasive stimulation with Temporal Interference: Optimization of the electric field deep in the brain with the use of a genetic algorithm. In *J. Neural Eng.* 19 (5), p. 56018. DOI: 10.1088/1741-2552/ac89b3.

Su, Xiaofan; Guo, Jiahui; Zhou, Meixuan; Chen, Jianpin; Li, Liming; Chen, Yao et al. (2021): Computational Modeling of Spatially Selective Retinal Stimulation With Temporally Interfering Electric Fields. In *IEEE transactions on neural systems and rehabilitation engineering* 29, pp. 418–428. DOI: 10.1109/tnsre.2021.3055203.

Sunshine, Michael D.; Cassarà, Antonino M.; Neufeld, Esra; Grossman, Nir; Mareci, Thomas H.; Otto, Kevin J. et al. (2021): Restoration of breathing after opioid overdose and spinal cord injury using temporal interference stimulation. In *Commun Biol* 4 (1), p. 107. DOI: 10.1038/s42003-020-01604-x.

Tadini, Laura; El-Nazer, Rasheda; Brunoni, Andre Russowsky; Williams, Julie; Carvas, Marcelo; Boggio, Paulo et al. (2011): Cognitive, mood, and electroencephalographic effects of noninvasive cortical stimulation with weak electrical currents. In *The Journal of ECT* 27 (2), pp. 134–140. DOI: 10.1097/YCT.0b013e3181e631a8.

Thiele, Carsten; Rufener, Katharina S.; Repplinger, Stefan; Zaehle, Tino; Ruhnau, Philipp (2024a): Transcranial temporal interference stimulation (tTIS) influences event-related alpha activity during mental rotation. In *Psychophysiology*, e14651. DOI: 10.1111/psyp.14651.

Thiele, Carsten; Tamm, Cornelius; Ruhnau, Philipp; Zaehle, Tino (2024b): Perceptibility and Pain Thresholds in Low- and High-Frequency Alternating Current Stimulation: Implications for tACS and tTIS. In *J Cogn Enhanc*, pp. 1–13. DOI: 10.1007/s41465-024-00304-2.

Thiele, Carsten; Zaehle, Tino; Haghikia, Aiden; Ruhnau, Philipp (2021): Amplitude modulated transcranial alternating current stimulation (AM-TACS) efficacy evaluation via phosphene induction. In *Sci Rep* 11 (1), p. 22245. DOI: 10.1038/s41598-021-01482-1.

Thielscher, A.; Antunes, A.; Saturnino, G. B. (2015): Field modeling for transcranial magnetic stimulation: a useful tool to understand the physiological effects of TMS? In *37th annual international conference of the IEEE engineering in medicine and biology society (EMBC)*. DOI: 10.1109/EMBC.2015.7318340.

Thut, Gregor; Miniussi, Carlo; Gross, Joachim (2012): The functional importance of rhythmic activity in the brain. In *Current biology : CB* 22 (16), R658-63. DOI: 10.1016/j.cub.2012.06.061.

Thut, Gregor; Schyns, Philippe G.; Gross, Joachim (2011): Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. In *Front. Psychol.* 2, p. 170. DOI: 10.3389/fpsyg.2011.00170.

Turi, Zs; Ambrus, G. G.; Janacsek, K.; Emmert, K.; Hahn, L.; Paulus, W.; Antal, A. (2013): Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation. In *Restorative neurology and neuroscience* 31 (3), pp. 275–285. DOI: 10.3233/RNN-120297.

Vieira, Pedro G.; Krause, Matthew R.; Pack, Christopher C. (2023): Temporal interference stimulation disrupts spike timing in the primate brain. DOI: 10.1101/2023.09.25.559340.

Violante, Ines R.; Alania, Ketevan; Cassarà, Antonino M.; Neufeld, Esra; Acerbo, Emma; Carron, Romain et al. (2023): Non-invasive temporal interference electrical stimulation of the human hippocampus. In *Nat Neurosci*, pp. 1–11. DOI: 10.1038/s41593-023-01456-8.

Vogeti, Sreekari; Boetzel, Cindy; Herrmann, Christoph S. (2022): Entrainment and spike-timing dependent plasticity–a review of proposed mechanisms of transcranial alternating current stimulation. In *Frontiers in Systems Neuroscience* 16. DOI: 10.3389/fnsys.2022.827353.

Volkmann, Jens (2004): Deep brain stimulation for the treatment of Parkinson's disease. In *Journal of clinical neurophysiology* 21 (1), pp. 6–17. DOI: 10.1097/00004691-200401000-00003.

Wang, Boshuo; Aberra, Aman S.; Grill, Warren M.; Peterchev, Angel V. (2022): Responses of Model Cortical Neurons to Temporal Interference Stimulation and Related Transcranial Alternating Current Stimulation Modalities: Cold Spring Harbor Laboratory.

Ward, Alex R. (2009): Electrical stimulation using kilohertz-frequency alternating current. In *Phys Ther* 89 (2), pp. 181–190. DOI: 10.2522/ptj.20080060.

Waza, M.; Hayashi, Y.; Sakurai, T.; Harada, N.; Nishida, H.; Isono, M.; Oku, Y. (2017): Efficacy of interferential currents stimulation on post-stroke dysphagia: A case control study. In *Journal of the Neurological Sciences* 381, p. 838. DOI: 10.1016/j.jns.2017.08.2358.

Wessel, Maximilian J.; Beanato, Elena; Popa, Traian; Windel, Fabienne; Vassiliadis, Pierre; Menoud, Pauline et al. (2023): Noninvasive theta-burst stimulation of the human striatum enhances striatal activity and motor skill learning. In *Nat Neurosci*, pp. 1–12. DOI: 10.1038/s41593-023-01457-7.

Zaehle, Tino; Rach, Stefan; Herrmann, Christoph S. (2010): Transcranial alternating current stimulation enhances individual alpha activity in human EEG. In *PloS one* 5 (11), e13766. DOI: 10.1371/journal.pone.0013766.

Zhang, Keying; Guo, Ling; Zhang, Junping; An, Guangzhou; Zhou, Yan; Lin, Jiajin et al. (2019): A safety study of 500 μA cathodal transcranial direct current stimulation in rat. In *BMC Neurosci* 20 (1), p. 40. DOI: 10.1186/s12868-019-0523-7.

Zhang, Yufeng; Zhou, Zhining; Zhou, Junhong; Qian, Zhenyu; Lü, Jiaojiao; Li, Lu; Liu, Yu (2022a): Temporal interference stimulation targeting right frontoparietal areas enhances working memory in healthy individuals. In *Front. Hum. Neurosci.* 16, p. 918470. DOI: 10.3389/fnhum.2022.918470.

Zhang, Zhao; Lin, Bor-Shing; Wu, Chun-Wei George; Hsieh, Tsung-Hsun; Liou, Jian-Chiun; Li, Yu-Ting; Peng, Chih-Wei (2022b): Designing and Pilot Testing a Novel Transcranial Temporal Interference Stimulation Device for Neuromodulation. In *IEEE transactions on neural systems and rehabilitation engineering* 30, pp. 1483–1493. DOI: 10.1109/tnsre.2022.3179537.

Zheng, Suwang; Fu, Tianli; Yan, Jinlong; Zhu, Chunyue; Li, Lu; Qian, Zhenyu et al. (2024): Repetitive temporal interference stimulation improves jump performance but not the postural stability in young healthy males: a randomized controlled trial. In *J NeuroEngineering Rehabil* 21 (1), p. 38. DOI: 10.1186/s12984-024-01336-7.

Zhu, Zhiqiang; Xiong, Yiwu; Chen, Yun; Jiang, Yong; Qian, Zhenyu; Lu, Jianqiang et al. (2022): Temporal Interference (TI) Stimulation Boosts Functional Connectivity in Human Motor Cortex: A Comparison Study with Transcranial Direct Current Stimulation (tDCS). In *Neural Plasticity* 2022, p. 7605046. DOI: 10.1155/2022/7605046.

4. Publications included in this Thesis

- Thiele, C., Zaehle, T., Haghikia, A., & Ruhnau, P. (2021). Amplitude modulated transcranial alternating current stimulation (AM-TACS) efficacy evaluation via phosphene induction. *Scientific reports*, *11*(1), 22245. https://doi.org/10.1038/s41598-021-01482-1

- Thiele, C., Rufener, K. S., Repplinger, S., Zaehle, T., & Ruhnau, P. (2024). Transcranial temporal interference stimulation (tTIS) influences event-related alpha activity during mental rotation. *Psychophysiology*, e14651. https://doi.org/10.1111/psyp.14651

- Thiele, C., Tamm, C., Ruhnau, P., & Zaehle, T. (2024). Perceptibility and Pain Thresholds in Low-and High-Frequency Alternating Current Stimulation: Implications for tACS and tTIS. *Journal of Cognitive Enhancement*, 1-13. https://doi.org/10.1007/s41465-024-0030

scientific reports

Check for updates

OPEN

Amplitude modulated transcranial alternating current stimulation (AM-TACS) efficacy evaluation via phosphene induction

Carsten Thiele[1,2]✉, Tino Zaehle[1,2], Aiden Haghikia[1,2,3] & Philipp Ruhnau[1,2]

Amplitude modulated transcranial alternating current stimulation (AM-tACS) is a novel method of electrostimulation which enables the recording of electrophysiological signals during stimulation, thanks to an easier removable stimulation artefact compared to classical electrostimulation methods. To gauge the neuromodulatory potential of AM-tACS, we tested its capacity to induce phosphenes as an indicator of stimulation efficacy. AM-tACS was applied via a two-electrode setup, attached on FpZ and below the right eye. AM-tACS waveforms comprised of different carrier (50 Hz, 200 Hz, 1000 Hz) and modulation frequencies (8 Hz, 16 Hz, 28 Hz) were administered with at maximum 2 mA peak-to-peak stimulation strength. TACS conditions in the same frequencies were used as a benchmark for phosphene induction. AM-tACS conditions using a 50 Hz carrier frequency were able to induce phosphenes, but with no difference in phosphene thresholds between modulation frequencies. AM-tACS using a 200 Hz or 1000 Hz carrier frequency did not induce phosphenes. TACS conditions induced phosphenes in line with previous studies. Stimulation effects of AM-tACS conditions were independent of amplitude modulation and instead relied solely on the carrier frequency. A possible explanation may be that AM-tACS needs higher stimulation intensities for its amplitude modulation to have a neuromodulatory effect.

Neuronal oscillations across a range of frequencies are the basis for communication in the brain and underly many cognitive functions[1]. Transcranial alternating current stimulation (tACS) allows us to modulate this oscillatory activity in the brain[2–6]. On single-neuron level, tACS causes shifts in frequency and phase of neuronal spike timing[7]. On a population level, it is thought that tACS leads to "entrainment", i.e., phase-locking of neuronal activity to the applied frequency[8]. When applied at a task-relevant frequency, tACS therefore can lead to observable changes in behaviour (and presumably change in underlying function), due to modulations of task-relevant brain oscillations[5]. Because of that, tACS has been used to research many different brain functions, including memory[9], motor performance[10], working memory[11], creative thinking[12] or motion perception[13], just to name a few.

Most tACS studies are focussing on the behavioural effects of stimulation[14–16], while they lack electrophysiological recordings to confirm the modulation of neuronal activity. This is due to a significant artefact to any electrophysiological recording caused by the electrostimulation which poses a challenge to analyse online-effects (i.e., effects during stimulation) of tACS[17]. Other studies bypass this problem by relying on offline-effects (i.e., effects after stimulation) of tACS. Previous studies were able to demonstrate modulations of endogenous oscillations after tACS which outlast the stimulation for up to 70 min[18] (for a review see[19]). However, offline-effects are likely not based on neuronal entrainment but rather on changes in neuronal plasticity, therefore it has been concluded that online- and offline-effects are qualitatively different[2].

To be able to study online-effects, it is necessary to analyse (artefact-free) brain activity recorded during stimulation. This has been proven very challenging, since signal subtraction approaches may be able to reduce the amplitude of linear stimulation artefacts by a significant amount[20], but they fail to eliminate nonlinear stimulation artefacts introduced by, for instance, broadband noise or interactions of physiological processes (e.g., respiratory-, cardiac- or oculomotoric activity) with the stimulation[17]. There is typically a substantial amount of residual artefact which might be mistaken as brain activity[21]. A further complicating fact is that the stimulation

[1]Department of Neurology, Otto-Von-Guericke-Universität, Universitätsklinikum Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany. [2]Center for Behavioral Brain Sciences (CBBS), Otto-Von-Guericke-Universität Magdeburg, 39120 Magdeburg, Germany. [3]German Center for Neurodegenerative Diseases, Magdeburg, Germany. ✉email: carsten.thiele@med.ovgu.de

frequency and neuronal frequency of interest are identical, making the use of spectral filters unfeasible to reduce artefact strength, since the brain activity of interest would be eliminated from the data as well. Therefore, more sophisticated methods are needed to recover the real brain signal from the overlapping stimulation artefact. One of the possible solutions that was found for this problem, was to apply spatial filters like beamforming[22,23]. But there is still some debate as to how effective these filters are at removing the stimulation artefacts, as well as non-linear interactions[17,24,25].

A different approach to circumvent the artefact problem was proposed by Witkowski et al.[26] who used amplitude modulated tACS (AM-tACS). This method uses a stimulation waveform that consists of two components: a high-frequency (> 150 Hz) sinusoidal carrier and a low-frequency (e.g., 10 Hz) amplitude modulation. When combined, the modulation signal leads to a sinusoidally rising and falling amplitude of the carrier signal, often referred to as the envelope, generating an amplitude modulated waveform. It is important to note that it is not the high-frequency carrier signal which stimulates neuronal activity, but rather the low-frequency amplitude modulation. Other stimulation methods which rely on the combination of a carrier signal and a stimulating component already exist, for instance, in the form of cross-frequency tACS[27] where typically a continuous low-frequency carrier is combined with a high-frequency signal aligned to a certain phase of the carrier (e.g., a 6 Hz carrier combined with short gamma-frequency bursts[28]). But a carrier frequency this low already has a stimulation effect by itself, which AM-tACS avoids by employing a carrier frequency that is too high to have a neurostimulatory effect. This is because of low-pass properties of neuronal membranes which attenuate high-frequency stimulation[29,30]. The amplitude modulation frequency, on the other hand, is chosen to be low enough to be able to entrain neuronal activity[31,32]. Recent work theoretically confirms the stimulation effect of the amplitude modulation and therefore the feasibility of AM-tACS, for instance, using modelling[33] or with hippocampal slices in-vitro[34]. Previous studies also found stimulation effects of AM-tACS in humans, as it e.g. disrupted performance in a working memory task[35] or affected visual perception[36].

AM-tACS aims to allow for the analysis of online stimulation effects, by theoretically avoiding the contamination of the recorded brain oscillations at the frequency of interest with a stimulation artefact[26]. When using AM-TACS, the recorded signal should only be contaminated by the carrier frequency, which is way beyond the frequency of interest. The frequency of the amplitude modulation on the other hand exhibits no spectral power[37], thus not introducing an artefact into the signal. As a result, AM-tACS—with an appropriate low pass filter to eliminate the carrier—should allow for stimulation while concurrently recording artefact-free brain signals at the frequency of interest, therefore making online effects observable. In practice however, recent studies were able to demonstrate that due to nonlinear transfer characteristics of stimulation and recording hardware, some artefacts are still introduced in the electrophysiological recording that have to be accounted for[37], but can be removed using noise reduction techniques[36].

Of note, a promising new type of transcranial electric stimulation using temporal interference[38] relies on a similar amplitude modulated signal. This method does not use an amplitude modulated signal that is emitted from the electrodes (which is the case with AM-tACS), but instead relies on two interfering alternating current fields. Due to the fact that the fields alternate with differing frequencies, they result in an amplitude modulated current at the point of interference, i.e., in the targeted brain area where the fields overlap. This way, this method may allow for non-invasive stimulation of deeper brain structures by generating a remote-target AM-tACS-like signal. Thus, evaluating AM-tACS can also help to advance our knowledge about the efficacy and mechanism of action of temporal interference stimulation.

In our study, we probed the efficacy of AM-tACS using visual phosphenes. Phosphenes are perceptions of flashing or shimmering light in the absence of accompanying visual input which can be easily induced by applying an electric current to the retina[39]. Since the eye, or rather the retina, can be considered part of the central nervous system (CNS) and a model of electric CNS circuitry, retinal phosphenes have been used as a robust indicator to gauge efficacy of CNS stimulation[40]. Though electrically induced phosphenes have initially thought to be created by visual cortex stimulation[41], it is now widely accepted that the electric current is travelling along the skin of the head to the eyes, causing retinal activation and thus making the retina the source of phosphenes[39,42–47]. In previous AM-TACS studies[35,36], behavioral effects have been found, but subjects reported having seen no phosphenes. This is likely due to electrode placements targeting the brain, leading to low current density at the retina. Therefore, in our study we optimized the electrode setup to increase electric currents reaching the retina in order to maximize the phosphene induction potential.

In our study, we examine the efficacy of AM-tACS (see Fig. 1a (Bottom)) stimulation on the CNS. This will be further evaluated by also applying classical tACS (see Fig. 1a (Top)) as a benchmark of stimulation efficacy. Phosphene thresholds (lowest needed stimulation intensity to induce phosphenes) will be used to compare stimulation efficacy of stimulation methods and -frequencies. In case of AM-tACS, we hypothesize that phosphene thresholds will overall vary between carrier frequencies, as the required stimulation intensity for an effective stimulation rises with higher carrier frequencies[33]. We further hypothesize that phosphene thresholds will vary within a carrier frequency, as a function of amplitude modulation frequency. This would prove the theorized stimulation effect of AM-tACS due to the amplitude modulation of its waveform[26]. We further hypothesize that tACS will induce phosphenes in line with prior studies[48], but also at lower needed stimulation intensities when compared to AM-tACS[33,34].

To this day, attempts at in-vivo stimulation with AM-tACS are scarce, and methodological difficulties when using this method remain[37]. This study therefore aims to advance the understanding of AM-tACS and to gain new insights into the feasibility of this method as a new neuromodulatory tool.

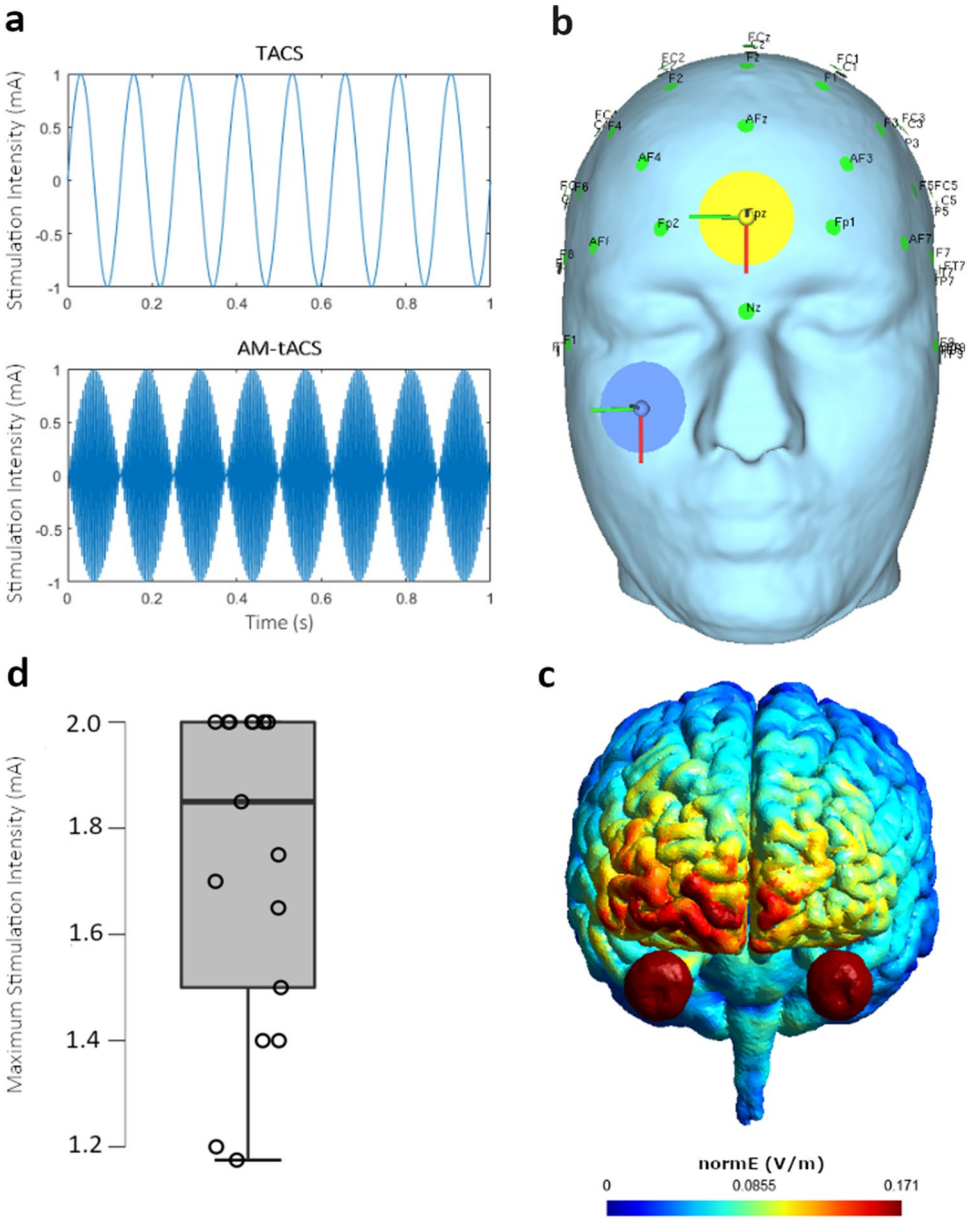

Figure 1. **(a)** Used stimulation methods exemplified. Top: TACS with 8 Hz. Bottom: AM-tACS with 8 Hz amplitude modulation frequency and 200 Hz carrier frequency. **(b)** Electrode setup used in this experiment with two 34 mm circular electrodes, placed at FpZ and on the cheek below the right eye. **(c)** Simulation of current distribution during 2 mA stimulation. Generated with SimNIBS [50]. **(d)** Distribution of subjects' individual maximum stimulation intensities as determined by the pain threshold procedure.

Material and methods

Subjects. 19 healthy subjects (14 female; age range: 19–29 years; mean age: 23.6 years) took part in the experiment. All subjects reported to be free of neurological illness, having no history of epileptic seizures, no metal or other medical implants in their body, had no uncorrected visual impairments and were not taking medication with an effect on the central nervous system. Prior to the experiment, subjects gave written informed consent after being informed about the experimental procedure as well as potential adverse effects of TES. Two subjects had to be excluded from data analysis, because in at least two conditions their phosphene thresholds were substantially higher than those conditions' mean phosphene thresholds (three standard deviations above the mean), possibly due to a problem with the attachment of the electrodes or non-compliance with the task. This left 17 subjects (12 female; age range: 19–29 years; mean age: 23.8 years) for the data analysis. This research was approved by the local ethics committee of the University Clinic of Magdeburg and carried out in accordance with the guidelines of the Declaration of Helsinki.

Design and staircase procedure. A trial consisted of three seconds of ramping the stimulation up, five seconds of stimulation[48], followed by one second of ramping down. A timer on the screen informed subjects when the stimulation was ramped up/down, as well as of the remaining duration of the stimulation. After the stimulation period, subjects answered a self-paced yes/no question if they saw phosphenes during stimulation (using their index and middle finger on the buttons 'J' and 'N' on a keyboard). Subjects were instructed to keep their eyes open at all times. After subjects gave their response, the next trial started. To avoid condition repetition effects, the order of conditions was pseudorandomized such that each trial was followed by a trial of another condition. This was done to balance carry-over effects across conditions.

Trials were embedded in a condition-wise staircase procedure which determined stimulation intensity for each trial based on prior given responses for trials of that condition. This was done to determine thresholds for phosphene perception for each condition, using a 1-up-1-down staircase procedure[49]. Depending on the subjects' answer for a trial, the stimulation intensity for its corresponding condition was adjusted, with an increase in stimulation intensity for future trials of that condition if no phosphenes were seen and vice versa. The rate of stimulation intensity adjustment was dependent on the number of reversals of answers. A reversal of answers was counted when a 'yes' (i.e., phosphenes were seen) answer to a condition was followed by a 'no' (i.e., no phosphenes were seen) answer or vice versa. In the beginning, at zero reversals, intensities were adjusted by 50% of the condition's stimulation intensity (e.g., a condition set to 1 mA was lowered to 0.5 mA for future trials if it was able to induce phosphenes), with each reversal lowering the adjustment rate by 5%. This meant that after each reversal, the intensity adjustment for a condition became incrementally smaller, decreasing from the initial 50% adjustment rate at the beginning, down to a 15% adjustment rate for trials after the 7th reversal. This allowed for quickly reaching the area of the threshold with larger steps at the beginning, as well as closing in on the precise threshold value with smaller steps at the end.

The staircase for a condition was concluded in one of three possible ways: (1) with the occurrence of an 8th reversal, (2) with the 20th trial in that condition, (3) with the condition running into its maximum intensity value for a 2nd time without phosphene percept. If the staircase concluded in the first or second way, the phosphene threshold was calculated as the mean of all intensities at reversals, excluding the first reversal. If it concluded in the third way, the phosphene threshold for that condition was set to the maximum of 2 mA for statistical analyses.

Every ten minutes of the experiment, subjects took a one-minute fixed break.

Stimulation. The transcranial electrostimulation was applied using a battery-driven stimulator system (DC-Stimulator Plus, NeuroConn GmbH, Ilmenau, Germany) operating in external mode. The stimulation was driven via a custom Matlab (Version 2019a, Mathworks, Natick, USA) script using the data acquisition toolbox and a digital/analog converter (DAC; NI USB-6212, National Instruments, Austin, TX, USA) connected to the REMOTE port of the stimulator.

For the electrode setup, two circular (34 mm diameter) carbon–rubber conductive electrodes (NeuroConn GmbH, Ilmenau, Germany) were used, with one electrode placed at FpZ and the second electrode vertically centered and approximately 2.5 cm below the right eye (see Fig. 1b). By using relatively small electrodes placed close to the eye, we were able to maximize the current that reaches the retina (for a simulation of current distribution see Fig. 1c). Electrodes were applied to the skin using conductive paste (Ten20, D.O. Weaver, Aurora, CO, USA) with impedances being kept below 5 kΩ.

In this experiment, the maximum stimulation intensity was determined individually for each subject by a pain threshold procedure. Pain thresholds were determined by manually increasing the stimulation intensity of a 200 Hz tACS (the condition at which pain sensations occurred most often), beginning at 0.5 mA peak-to-peak and increasing in steps of 0.25 mA, until subjects either reported adverse side effects (pain sensations or uncomfortable tingling at the electrodes), or the maximum stimulation intensity of 2 mA peak-to-peak was reached. This pain threshold was set as the maximum stimulation intensity for all stimulation conditions except the 1000 Hz conditions (whose possible maximum stimulation intensity was always set to 2 mA), as stimulation with frequencies this high does not induce pain sensations.

Sinusoidal stimulations were applied with no DC offset. Due to a difference in expected phosphene thresholds for tACS and AM-tACS, initial stimulation intensities (i.e., intensities at which the respective staircases started) were set to 0.15 mA for tACS conditions and 1 mA for AM-tACS conditions.

For AM-tACS, the signal was computed based on the following equation:

$$AM_{Signal}(t) = a_{stim} * \frac{\left(\sin\left(2\pi * f_c * t\right) + \sin\left(2\pi * \left(f_c + f_m\right) * t\right)\right)}{2}$$

where t is the time course, a_{stim} the amplitude of the sine wave, f_c the frequency of the carrier and f_m the frequency of the amplitude modulation. The equation is taken from temporal interference studies[38] instead of AM-tACS studies[26] to allow us to also draw conclusions from the results to temporal interference stimulation. An amplitude modulated signal generated via temporal interference differs slightly from AM-tACS waveforms as its envelope is non-sinusoidal (sharp instead of round troughs). This unlikely affected our results, since it only causes a minor difference between the waveforms. To our knowledge there are no studies that make a statement about possible differences in stimulation effects due to the different shape of the waveforms; quite the opposite, they are usually assumed to work in similar ways. Only Kasten et al.[37] did a direct comparison between both waveforms, but only to confirm that both waveforms were inducing similar artefacts, without making a statement about differing stimulation mechanisms.

The amplitude modulation frequencies (8 Hz, 16 Hz, 28 Hz) were chosen based on tACS findings[48], to have one frequency (16 Hz) with a very low phosphene threshold, i.e. that optimally induces phosphenes at low stimulation intensity, as well as two less-optimal phosphene inducing frequencies (8 Hz, 28 Hz), with phosphene

thresholds matching each other. As for carrier frequencies (50 Hz, 200 Hz, 1000 Hz), one frequency (50 Hz) was chosen to be low enough to induce phosphenes regardless of amplitude modulation, another carrier frequency (200 Hz) was chosen based on an AM-tACS study[36], while the third (1000 Hz) was based on a study using temporal interference stimulation[38]. This resulted in a combination of 9 different stimulation patterns for AM-tACS (50×8, 50×16, 50×28, 200×8, 200×16, 200×28, 1000×8, 1000×16, 1000×28).

All amplitude modulation and carrier frequencies which were used for AM-tACS, were also administered using tACS (8 Hz, 16 Hz, 28 Hz, 50 Hz, 200 Hz, 1000 Hz). This was done to have tACS as a benchmark to (1) compare stimulation efficacy of amplitude modulation frequencies, when used with tACS vs AM-tACS, (2) to test if carrier frequencies themselves were able to induce phosphenes and (3) to disentangle the effects of amplitude modulation and carrier frequency on phosphene induction.

In order to detect subjects' tendency for false positive responses (i.e., answering 'yes' to having seen phosphenes without actual phosphene perception), three control conditions were included. The first was a sham condition without stimulation. In addition, controls using a transcranial direct current stimulation (tDCS) with anodal (anode above and cathode below the eye) and cathodal (cathode above and anode below the eye) stimulation were used to detect subjects who based their answer on skin sensations caused by the stimulation rather than on phosphene perceptions. The sham stimulation was set to 0 mA. The initial stimulation intensity for the tDCS controls was set to 0.1 mA with a maximum of 0.5 mA.

Procedure. Subjects were seated in a chair in front of a grey screen (RGB: 50 50 50) in a dimly lit room. After the stimulation was set up, individual pain thresholds were determined for each subject. Following that, subjects were familiarized with phosphene perception by administering a 16 Hz tACS with a stimulation intensity of 0.5 mA. Subjects were asked to verbally describe the shape of perceived phosphenes and the affected visual field, with all subjects reporting flashing lights in the right visual field. Following that, the task was explained to the subjects: after receiving stimulation, subjects were instructed to indicate if they saw phosphenes. At the beginning, subjects performed a training version of the task (5 trials) where 16 Hz tACS using differing intensities (0 mA, 0.125 mA, 0.25 mA, 0.375 mA, 0.5 mA) in a randomized order was administered. If no questions remained, the experiment began, taking approximately 30 min. Afterwards, subjects were reimbursed for their participation either with money or with course credit.

Statistical analysis. Statistical analyses were performed using SPSS Statistics v22 (IBM, Chicago, IL). Mean phosphene thresholds for each condition were calculated across all 17 subjects and examined using separate repeated measures analyses of variance (ANOVA) for tACS and AM-tACS.

A Shapiro–Wilk test (due to its high power compared to other normal-distribution tests[51,52]) was used to analyse the normal distribution assumption for all conditions that were able to induce phosphenes (TACS: 8 Hz, 16 Hz, 28 Hz, 50 Hz; AM-tACS: 50×8, 50×16, 50×28), with results indicating that all phosphene-inducing conditions except the 8 Hz ($W(17)=0.852$, $p=0.011$) and 16 Hz ($W(17)=0.784$, $p=0.001$) tACS conditions were normally-distributed. Non-phosphene-inducing conditions (TACS: 200 Hz, 1000 Hz; AM-tACS: 200×8, 200×16, 200×28, 1000×8, 1000×16, 1000×28) were not normally-distributed because they were set to maximum stimulation intensity of 2 mA for statistical analysis as they failed to induce phosphenes and hence showed no variance.

To analyse tACS conditions, a Friedman ANOVA was performed for the factor Stimulation Frequency (8 Hz, 16 Hz, 28 Hz, 50 Hz, 200 Hz, 1000 Hz). A Friedman ANOVA was chosen in this case due to some tACS conditions (200 Hz, 1000 Hz) not being normally distributed. As tACS conditions included only one within-subject factor, the Friedman ANOVA is a suitable non-parametric alternative.

For AM-tACS analysis, a non-parametric ANOVA would be preferable as well due to non-normally distributed AM-tACS conditions, but a non-parametrical approach is not feasible in this case due to more than one within-subject factor. Furthermore, studies argue that the repeated-measures ANOVA is sufficiently robust against non-normal distribution[53–55]. Therefore, AM-tACS conditions were analysed using a repeated-measures ANOVA with the factors Carrier Frequency (50 Hz, 200 Hz, 1000 Hz) and Amplitude Modulation Frequency (8 Hz, 16 Hz, 28 Hz).

Further, to directly compare stimulation methods and analyse differences between tACS and AM-tACS, a two-way repeated measures ANOVA with the factors Stimulation Method (TACS, AM-tACS) and Stimulation Frequency (8 Hz, 16 Hz, 28 Hz) was performed.

Mauchly's test of sphericity was used to ensure that sphericity could be assumed for all factors, with none violating this assumption. Significant main effects and interactions were followed up using Bonferroni-adjusted post-hoc tests. As an effect size measure, partial eta squared (η_p^2) is reported for repeated-measures ANOVAs.

For non-significant effects, repeated measures analyses were performed using a Bayesian framework in JASP (JASP Team, Version 0.14.1, 2020) to dissociate the lack of a statistical effect from poor sensitivity to uncover such an effect. Using Bayes analysis, a likelihood ratio of two competing hypotheses – the null hypothesis (H0) and an alternative hypothesis (H1) – is expressed using the factor BF_{10} (probability of the H1 over the H0) or alternatively using the BF_{01} (probability of the H0 over the H1). Bayes factors can range from 0 to infinity, with higher values indicating more support for the hypothesis. For instance, $BF_{10}=2$ indicates that the alternative hypothesis is twice as likely as the null hypothesis. For interpretation, a Bayes factor between 1 and 3 is considered weak evidence, up to 10 is considered moderate evidence and Bayes factors above 10 are considered strong evidence[56].

Results

All subjects tolerated the stimulation and no serious adverse-effects were reported after the experiment. The only adverse effect reported by subjects was an uncomfortable tingling sensation during stimulation. None of the 17 subjects reported seeing phosphenes during the sham or tDCS-control conditions. All subjects were

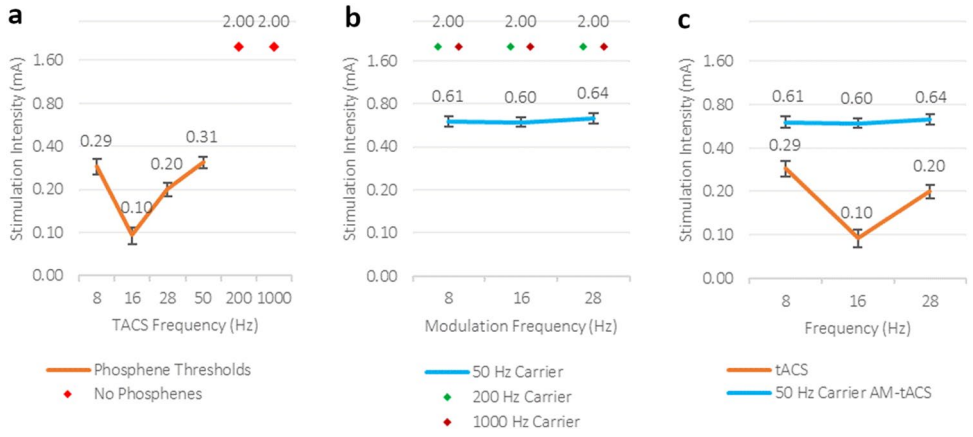

Figure 2. **(a)** Phosphene thresholds for all tACS conditions (8 Hz, 16 Hz, 28 Hz, 50 Hz, 200 Hz, 1000 Hz). Conditions form a V-shaped relation, with 16 Hz needing the least stimulation intensity to induce phosphenes. The 200 Hz and 1000 Hz stimulation did not induce phosphenes and were therefore set to the maximum stimulation intensity of 2 mA. **(b)** Phosphene thresholds for all AM-tACS conditions; 50 Hz carrier conditions (50 × 8, 50 × 16, 50 × 28), 200 Hz carrier conditions (200 × 8, 200 × 16, 200 × 28) and 1000 Hz carrier conditions (1000 × 8, 1000 × 16, 1000 × 28). Phosphene thresholds for the 50 Hz carrier conditions were similar across modulation frequencies. None of the 200 Hz and 1000 Hz carrier conditions induced phosphenes and therefore reached the maximum stimulation intensity of 2 mA. **(c)** Phosphene thresholds for AM-tACS modulation frequencies of 50 Hz carrier conditions (50 × 8, 50 × 16, 50 × 28) and corresponding tACS conditions (8 Hz, 16 Hz, 28 Hz).

able to tolerate maximum stimulation intensities above 1 mA [$M = 1.74$ mA, $SD = 0.30$ mA, $Min = 1.175$ mA, $Max = 2.00$ mA] with n = 8 subjects tolerating the maximum stimulation intensity of 2 mA (see Fig. 1d).

TACS. A Friedman's ANOVA for tACS with the factor Stimulation Frequency (8 Hz, 16 Hz, 28 Hz, 50 Hz, 200 Hz, 1000 Hz) revealed a statistically significant main effect [χ^2 (5, N = 17) = 78.875, $p < 0.001$]. Bonferroni-adjusted post-hoc analysis using the Conover Test revealed 16 Hz stimulation [$M = 0.10$ mA, $SD = 0.05$ mA] having significantly the lowest phosphene threshold, i.e., needing the least stimulation intensity to induce phosphenes, compared to all other frequencies except 28 Hz [$p_{corr} < 0.012$]. This was followed by the phosphene thresholds for 28 Hz [$M = 0.20$ mA, $SD = 0.09$ mA], 8 Hz [$M = 0.29$ mA, $SD = 0.15$ mA] and 50 Hz [$M = 0.31$ mA, $SD = 0.11$ mA] which differed not significantly from each other [$p_{corr} = 1$] but were significantly lower than the 200 Hz [$M = 2.00$ mA, $SD = 0.00$ mA] and 1000 Hz [$M = 2.00$ mA, $SD = 0.00$ mA] conditions [$p_{corr} < 0.033$] (see Fig. 2a). Due to 200 Hz and 1000 Hz conditions not inducing phosphenes and therefore being set to 2 mA, they did not differ significantly from each other [$p_{corr} = 1$] (see Supplementary Fig. S1 for single subject data of tACS conditions).

AM-tACS. A repeated-measures ANOVA with the factors Carrier Frequency (50 Hz, 200 Hz, 1000 Hz) and Amplitude Modulation Frequency (8 Hz, 16 Hz, 28 Hz) revealed a significant main effect of Carrier Frequency [$F(2,32) = 782.59$, $p < 0.001$, $\eta_p^2 = 0.98$], but neither the main effect of Amplitude Modulation Frequency [$F(2,32) = 1.11$, $p = 0.34$, $\eta_p^2 = 0.07$] nor the interaction between both factors [$F(4,64) = 1.11$, $p = 0.36$, $\eta_p^2 = 0.07$] were significant. Bonferroni-adjusted post-hoc analysis revealed a significant difference between the 50 Hz carrier frequency [$M = 0.61$ mA, $SD = 0.20$] and 200 Hz [$M = 2.00$ mA, $SD = 0.00$ mA] as well as 1000 Hz [$M = 2.00$ mA, $SD = 0.00$ mA] carrier frequencies [$p_{corr} < 0.001$] (see Fig. 2b). No difference between 200 and 1000 Hz carrier frequencies were found [$p_{corr} = 1$], due to no phosphenes being induced in these conditions and their phosphene thresholds reaching the maximum stimulation intensity of 2 mA (see Supplementary Fig. S2 for single subject data of AM-tACS conditions). Using a Bayesian repeated measures ANOVA, there is decisive evidence in favor of an effect of the carrier frequency ($BF_{10} = 3.912*10^{114}$), but also strong evidence for an absence of effect for the amplitude modulation ($BF_{01} = 15.615$).

To analyse if amplitude modulation frequencies can affect the phosphene threshold of the 50 Hz carrier stimulations (the only AM-tACS conditions with obtainable phosphene thresholds), we exploratively compared the 50 Hz carrier conditions 50 × 8 [$M = 0.61$ mA, $SD = 0.22$ mA], 50 × 16 [$M = 0.60$ mA, $SD = 0.18$ mA] and 50 × 28 [$M = 0.64$ mA, $SD = 0.23$ mA], revealing no differences between phosphene thresholds in these conditions [$p_{corr} > 0.662$]. Bayesian analysis revealed moderate evidence for an absence of effect for the amplitude modulation in 50 Hz carrier conditions ($BF_{01} = 3.06$).

TACS vs. AM-tACS. To analyse the differences in phosphene induction between tACS and AM-tACS, the AM-tACS conditions that were able to induce phosphenes (50 × 8, 50 × 16, 50 × 28) were compared to the corresponding tACS conditions (8 Hz, 16 Hz, 28 Hz) (see Fig. 2c) using a repeated measures ANOVA with the factors Stimulation Method (tACS, AM-tACS) and Stimulation Frequency (8 Hz, 16 Hz, 28 Hz). This revealed a signifi-

cant main effect of Stimulation Method [$F(1,16) = 121.02$, $p < 0.001$, $\eta_p^2 = 0.88$] due to lower phosphene thresholds in the tACS [$M = 0.20$ mA, $SD = 0.09$ mA], compared to the AM-tACS [$M = 0.61$ mA, $SD = 0.20$ mA] conditions.

Furthermore, the Stimulation Frequency main effect was significant [$F(2,32) = 14.17$, $p < 0.001$, $\eta_p^2 = 0.47$] but could be explained by the significant interaction between both factors [$F(2,32) = 16.94$, $p < 0.001$, $\eta_p^2 = 0.51$]. This interaction was due to significant differences between frequencies for tACS conditions [$p_{corr} < 0.035$] (see tACS section above), but not for AM-tACS conditions [$p_{corr} > 0.662$] (see AM-tACS section above).

It should be noted, when using 50 Hz as a carrier frequency [$M = 0.61$ mA, $SD = 0.20$] it needs around double the intensity [$t(16) = 10.75$, $p < 0.001$] to elicit phosphenes as compared to when 50 Hz is used as a tACS frequency [$M = 0.31$ mA, $SD = 0.11$], because 50 Hz AM-tACS, over time, is reduced in amplitude compared to tACS by a factor of 2 due to the amplitude modulation.

Discussion

In this study we evaluated the efficacy of AM-tACS to gauge its potential as a neuromodulatory tool. To this end, we applied AM-tACS as well as tACS at different stimulation frequencies to the retina of the right eye to induce phosphenes. Our results show that, while tACS clearly induces phosphenes in line with previous studies[48,57], the amplitude modulation of sine waves, which is the key stimulation mechanism of AM-tACS[26], did not induce phosphenes. This indicates that AM-tACS does not sufficiently stimulate neurons in the retina which questions its use as a non-invasive neuromodulatory tool.

While the stimulation of the CNS using tACS is a method which has garnered much attention in the last years[2,58–60], a recent variation of tACS, which uses amplitude modulated sine-waves (AM-tACS)[26], is only scarcely researched. Thus, in this study, we aimed to evaluate AM-tACS in respect to its neuromodulatory potential and examined the influence of carrier frequency and amplitude modulation frequency, of which the AM-tACS waveform is comprised of. For this, we used the retina as a model for CNS circuitry and phosphenes as an indicator for stimulation efficacy[40,61]. We attached electrodes around the right eye of subjects and applied AM-tACS at different carrier- and amplitude modulation frequencies. In addition, to serve as a benchmark, we applied regular tACS at the same frequencies as well.

For tACS, this resulted in a replication of known interactions[48,57] between stimulation frequency and needed stimulation intensity to reach the phosphene threshold (the lowest intensity at which phosphenes were induced). The 16 Hz stimulation induced phosphenes most readily and needed the least stimulation intensity to reach its phosphene threshold. This was followed by 28 Hz needing around double the stimulation intensity, as well as 8 Hz and 50 Hz needing three times the intensity compared to 16 Hz stimulation to induce phosphenes. Stimulation with even higher frequencies of 200 Hz and 1000 Hz did not induce phosphenes. This was expected due to the low-pass nature of neurons which greatly reduces the stimulation effectiveness[29].

For AM-tACS, this low-pass nature of neurons is circumvented by relying not on the high frequency of the sine wave (the carrier frequency) but rather on a much lower-frequency amplitude modulation (modulation frequency) of the sine wave, with the latter being the source of stimulation effects[34]. However, our results show that, within typical tACS intensity limits of 2 mA peak-to-peak, it is not possible to induce phosphenes via the modulation frequency. None of the AM-tACS conditions using 200 Hz or 1000 Hz carrier frequencies were able to induce phosphenes. The only AM-tACS conditions with obtainable phosphene thresholds were conditions using a 50 Hz carrier. However, when comparing tACS at 50 Hz and AM-tACS with a 50 Hz carrier, it became clear that the induced phosphenes in these conditions were not influenced by the amplitude modulation, but rather solely relied on the carrier frequency. In addition, a direct comparison between amplitude modulation frequencies (using the 50 Hz carrier) did not reveal differences in phosphene thresholds.

Furthermore, our results revealed that 50 Hz AM-tACS conditions need around double the stimulation intensity compared to 50 Hz tACS to induce phosphenes. This was expected, since the amplitude modulation reduces the net stimulation intensity to about half. This is a limitation of all studies using amplitude modulated signals and should be considered when interpreting results, as a non-amplitude-modulated signal (e.g., with 1 mA) is only comparable to an amplitude modulated signal with double the intensity (e.g., 2 mA). Consequently, we need to assume that phosphenes in the AM-tACS conditions were only due to the carrier frequency. Thus, it can be concluded that modulation frequencies had no stimulatory effect on the retina and did not induce phosphenes.

There are stimulation techniques, such as transcranial magnetic stimulation (TMS) or electric deep brain stimulation (DBS) which are strong enough to trigger action potentials in stimulated brain cells, thus achieving supra-threshold stimulation. In contrast, non-invasive transcranial electric stimulation techniques such as tACS are generally not strong enough to trigger action potentials. Rather, by using mechanisms such as entrainment or causing changes in synaptic plasticity, tACS uses sub-threshold modulation to facilitate or inhibit a neuron's likelihood to fire, therefore biasing neuronal activity[62–64]. Phosphenes are a rare example, because they manage to bridge the gap between non-invasive electrostimulation and supra-threshold stimulation, as the cells of the retina are sensitive enough to be triggered by low electric currents. This allows for an easily attainable and reliable readout of stimulation effects.

Though phosphenes have been extensively used as an indicator of neural activation[40,57,61], it is still not well understood how they are elicited by electric stimulation. As it was first believed that (non-invasively) electrically induced phosphenes were due to direct cortical activation[41], further studies showed that phosphenes are retinal (and not cortical) in origin[42–47]. For instance, a study from Kar and Krekelberg[47] debates that stimulation of axons of the retinal ganglion cells are a possible source for phosphenes. The results of this study are supported by a study from Delbeke et al.[65] who induced phosphenes via direct optic nerve stimulation. This is also in line with studies that show that especially axon terminals are susceptible to electric stimulation[66]. A modeling study[34] confirmed that the sensitivity to amplitude modulated stimulation using high-frequency carriers is dependent on a fast membrane time constant (i.e., the amount of time it takes for a change in potential from initial value to

63% of its final value). As axons have a very fast membrane time constant[34], the induction of phosphenes with AM-tACS via axonal stimulation should be theoretically possible.

A limitation of this approach (and therefore our findings) is its generalizability, because stimulation efficacy measures are not directly translatable from retinal cells to brain cells, since stimulation is achieved by different mechanisms. However, a number of studies have shown that tACS causes modulation in brain cells (via sub-threshold modulation using entrainment and changes in synaptic plasticity) as well as an activation of retinal cells (via supra-threshold stimulation), causing an induction of phosphenes. Therefore, it is possible to observe a tACS effect by measuring either of those. Hence, in our study we aimed to establish this link between retinal and brain activation for AM-tACS. However, our results question AM-tACS' efficacy in retinal stimulation because no supra-threshold stimulation could be achieved. However, this does not rule out potential sub-threshold effects of AM-tACS. Further research in this domain is therefore required.

The question remains why AM-tACS in our study failed to induce phosphenes. One possible explanation for that might be that the intensity of AM-tACS we used was too low to induce phosphenes. Modeling studies have estimated that the needed stimulation intensity of amplitude modulated sine waves has to be multiple times higher than with unmodulated sine waves, to yield comparable stimulation effects[33]. In an empirical study, Esmaeilpour et al.[34] applied AM-tACS to rodent hippocampal brain slices to show a modulation of neuronal firing during stimulation, but with considerably more stimulation intensity needed than with tACS (at least 167 mA with AM-tACS compared to 2 mA with tACS). As we applied stimulation intensities with a maximum of 2 mA in this study, this may explain why no phosphenes could be induced. One could consider using higher stimulation intensities for AM-tACS, however this would require higher carrier frequencies (which induce less skin sensations), since only half of our subjects were able to tolerate the maximum stimulation intensity of 2 mA without pain sensations when using 200 Hz carriers. But as studies have shown, there exists a trade-off between higher carrier frequencies also needing higher stimulation intensities for comparable effects[34]. Applying higher stimulation intensities is therefore not trivial, since one of the most important features of (AM-)tACS is its (pain-free) non-invasiveness, which cannot just be solved by increasing the carrier frequency.

As, due to reasons discussed here, we were not able to observe supra-threshold activation, future research has to determine if AM-tACS can cause sub-threshold modulations of neural activity instead.

Conclusion

In this study we evaluated the efficacy of AM-tACS as a neuromodulatory tool, by inducing phosphenes which served as an indicator of neuronal activation. Using a two-electrode setup around the right eye and different carrier- and modulation frequencies, we show that AM-tACS with stimulation intensities of up to 2 mA peak-to-peak is not able to induce phosphenes. While ultimately we did not induce phosphenes using AM-tACS, this study may serve as the basis for future studies that aim to better understand the mechanisms and efficacy of AM-tACS.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 1 July 2021; Accepted: 18 October 2021
Published online: 15 November 2021

References

1. Başar, E., Başar-Eroglu, C., Karakaş, S. & Schürmann, M. Gamma, alpha, delta, and theta oscillations govern cognitive processes. *Int. J. Psychophysiol.* **39**, 241–248 (2001).
2. Vosskuhl, J., Strüber, D. & Herrmann, C. S. Non-invasive brain stimulation: A paradigm shift in understanding brain oscillations. *Front. Hum. Neurosci.* **12**, 211 (2018).
3. Herrmann, C. S. & Strüber, D. What can transcranial alternating current stimulation tell us about brain oscillations?. *Curr. Behav. Neurosci. Rep.* **4**, 128–137 (2017).
4. Paulus, W., Nitsche, M. A. & Antal, A. Application of transcranial electric stimulation (tDCS, tACS, tRNS): From motor-evoked potentials towards modulation of behaviour. *Eur. Psychol.* **21**, 4 (2016).
5. Herrmann, C. S., Rach, S., Neuling, T. & Strüber, D. Transcranial alternating current stimulation: A review of the underlying mechanisms and modulation of cognitive processes. *Front. Hum. Neurosci.* **7**, 279 (2013).
6. Herrmann, C. S., Strüber, D., Helfrich, R. F. & Engel, A. K. EEG oscillations: From correlation to causality. *Int. J. Psychophysiol.* **103**, 12–21 (2016).
7. Reato, D., Rahman, A., Bikson, M. & Parra, L. C. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. *J. Neurosci.* **30**, 15067–15079 (2010).
8. Reato, D., Rahman, A., Bikson, M. & Parra, L. C. Effects of weak transcranial alternating current stimulation on brain activity—A review of known mechanisms from animal studies. *Front. Hum. Neurosci.* **7**, 687 (2013).
9. Vosskuhl, J., Huster, R. J. & Herrmann, C. S. Increase in short-term memory capacity induced by down-regulating individual theta frequency via transcranial alternating current stimulation. *Front. Hum. Neurosci.* **9**, 257 (2015).
10. Joundi, R. A., Jenkinson, N., Brittain, J.-S., Aziz, T. Z. & Brown, P. Driving oscillatory activity in the human cortex enhances motor performance. *Curr. Biol.* **22**, 403–407 (2012).
11. Polanía, R., Nitsche, M. A., Korman, C., Batsikadze, G. & Paulus, W. The importance of timing in segregated theta phase-coupling for cognitive performance. *Curr. Biol.* **22**, 1314–1318 (2012).
12. Lustenberger, C., Boyle, M. R., Foulser, A. A., Mellin, J. M. & Fröhlich, F. Functional role of frontal alpha oscillations in creativity. *Cortex* **67**, 74–82 (2015).
13. Kar, K. & Krekelberg, B. Transcranial alternating current stimulation attenuates visual motion adaptation. *J. Neurosci.* **34**, 7334–7340 (2014).
14. Rufener, K. S., Zaehle, T., Oechslin, M. S. & Meyer, M. 40Hz-Transcranial alternating current stimulation (tACS) selectively modulates speech perception. *Int. J. Psychophysiol.* **101**, 18–24 (2016).

15. Marshall, L., Helgadóttir, H., Mölle, M. & Born, J. Boosting slow oscillations during sleep potentiates memory. *Nature* **444**, 610–613 (2006).
16. Riecke, L., Formisano, E., Herrmann, C. S. & Sack, A. T. 4-Hz transcranial alternating current stimulation phase modulates hearing. *Brain Stimul.* **8**, 777–783 (2015).
17. Noury, N., Hipp, J. F. & Siegel, M. Physiological processes non-linearly affect electrophysiological recordings during transcranial electric stimulation. *Neuroimage* **140**, 99–109 (2016).
18. Kasten, F. H., Dowsett, J. & Herrmann, C. S. Sustained aftereffect of α-tACS lasts up to 70 min after stimulation. *Front. Hum. Neurosci.* **10**, 245 (2016).
19. Veniero, D., Vossen, A., Gross, J. & Thut, G. Lasting EEG/MEG aftereffects of rhythmic transcranial brain stimulation: Level of control over oscillatory network activity. *Front. Cell. Neurosci.* **9**, 477 (2015).
20. Voss, U. *et al*. Induction of self awareness in dreams through frontal low current stimulation of gamma activity. *Nat. Neurosci.* **17**, 810–812 (2014).
21. Gebodh, N. *et al.* Inherent physiological artifacts in EEG during tDCS. *Neuroimage* **185**, 408–424 (2019).
22. Neuling, T. *et al.* Friends, not foes: Magnetoencephalography as a tool to uncover brain dynamics during transcranial alternating current stimulation. *Neuroimage* **118**, 406–413 (2015).
23. Soekadar, S. R. *et al.* In vivo assessment of human brain oscillations during application of transcranial electric currents. *Nat Commun* **4**, 1–10 (2013).
24. Kasten, F. H. & Herrmann, C. S. Recovering brain dynamics during concurrent tACS-M/EEG: An overview of analysis approaches and their methodological and interpretational pitfalls. *Brain Topogr.* **32**, 1–7 (2019).
25. Noury, N. & Siegel, M. Phase properties of transcranial electrical stimulation artifacts in electrophysiological recordings. *Neuroimage* **158**, 406–416 (2017).
26. Witkowski, M. *et al.* Mapping entrained brain oscillations during transcranial alternating current stimulation (tACS). *Neuroimage* **140**, 89–98 (2016).
27. Riddle, J., McFerren, A. & Frohlich, F. Causal role of cross-frequency coupling in distinct components of cognitive control. *Prog. Neurobiol.* **202**, 102033 (2021).
28. Alekseichuk, I., Turi, Z., Amador de Lara, G., Antal, A. & Paulus, W. Spatial working memory in humans depends on theta and high gamma synchronization in the prefrontal cortex. *Current Biol.* **26**, 1513–1521 (2016).
29. Deans, J. K., Powell, A. D. & Jefferys, J. G. R. Sensitivity of coherent oscillations in rat hippocampus to AC electric fields. *J. Physiol.* **583**, 555–565 (2007).
30. Esmaeilpour, Z. *et al.* Limited sensitivity of hippocampal synaptic function or network oscillations to unmodulated kilohertz electric fields. *eNeuro* **7** (2020).
31. Beason, R. C. & Semm, P. Responses of neurons to an amplitude modulated microwave stimulus. *Neurosci. Lett.* **333**, 175–178 (2002).
32. Middleton, J. W., Longtin, A., Benda, J. & Maler, L. The cellular basis for parallel neural transmission of a high-frequency stimulus and its low-frequency envelope. *Proc. Natl. Acad. Sci.* **103**, 14596–14601 (2006).
33. Negahbani, E., Kasten, F. H., Herrmann, C. S. & Fröhlich, F. Targeting alpha-band oscillations in a cortical model with amplitude-modulated high-frequency transcranial electric stimulation. *Neuroimage* **173**, 3–12 (2018).
34. Esmaeilpour, Z., Kronberg, G., Reato, D., Parra, L. C. & Bikson, M. Temporal interference stimulation targets deep brain regions by modulating neural oscillations. *Brain Stimul.* **14**, 55–65 (2021).
35. Chander, B. S. *et al.* tACS phase locking of frontal midline theta oscillations disrupts working memory performance. *Front. Cell. Neurosci.* **10**, 120 (2016).
36. Minami, S. & Amano, K. Illusory jitter perceived at the frequency of alpha oscillations. *Curr. Biol.* **27**, 2344-2351.e4 (2017).
37. Kasten, F. H., Negahbani, E., Fröhlich, F. & Herrmann, C. S. Non-linear transfer characteristics of stimulation and recording hardware account for spurious low-frequency artifacts during amplitude modulated transcranial alternating current stimulation (AM-tACS). *Neuroimage* **179**, 134–143 (2018).
38. Grossman, N. *et al.* Noninvasive deep brain stimulation via temporally interfering electric fields. *Cell* **169**, 1029–1104 (2017).
39. Lindenblatt, G. & Silny, J. Electrical phosphenes: On the influence of conductivity inhomogeneities and small-scale structures of the orbita on the current density threshold of excitation. *Med. Biol. Eng. Compu.* **40**, 354–359 (2002).
40. Attwell, D. Interaction of low frequency electric fields with the nervous system: the retina as a model system. *Radiat. Prot. Dosimetry.* **106**, 341–348 (2003).
41. Kanai, R., Chaieb, L., Antal, A., Walsh, V. & Paulus, W. Frequency-dependent electrical stimulation of the visual cortex. *Curr. Biol.* **18**, 1839–1843 (2008).
42. Schwiedrzik, C. M. Retina or visual cortex? The site of phosphene induction by transcranial alternating current stimulation. *Front. Integr. Neurosci.* **3**, 6 (2009).
43. Schutter, D. J. L. G. & Hortensius, R. Retinal origin of phosphenes to transcranial alternating current stimulation. *Clin. Neurophysiol.* **121**, 1080–1084 (2010).
44. Paulus, W. On the difficulties of separating retinal from cortical origins of phosphenes when using transcranial alternating current stimulation (tACS). *Clin. Neurophysiol.* **121**, 987–991 (2010).
45. Evans, I. D., Palmisano, S. & Croft, R. J. Retinal and cortical contributions to phosphenes during transcranial electrical current stimulation. *Bioelectromagnetics* **42**, 146–158 (2021).
46. Laakso, I. & Hirata, A. Computational analysis shows why transcranial alternating current stimulation induces retinal phosphenes. *J. Neural Eng.* **10**, 46009 (2013).
47. Kar, K. & Krekelberg, B. Transcranial electrical stimulation over visual cortex evokes phosphenes with a retinal origin. *J. Neurophysiol.* **108**, 2173–2178 (2012).
48. Evans, I. D., Palmisano, S., Loughran, S. P., Legros, A. & Croft, R. J. Frequency-dependent and montage-based differences in phosphene perception thresholds via transcranial alternating current stimulation. *Bioelectromagnetics* **40**, 365–374 (2019).
49. Cornsweet, T. N. The staircase-method in psychophysics. *Am. J. Psychol.* **75**, 485–491 (1962).
50. Thielscher, A., Antunes, A. & Saturnino, G. B. Field modeling for transcranial magnetic stimulation: A useful tool to understand the physiological effects of TMS? *2015 37th annual international conference of the IEEE engineering in medicine and biology society (EMBC)* **2015,** 222–225 (2015).
51. Razali, N. M. & Wah, Y. B. Power comparisons of shapiro-wilk, kolmogorov-smirnov, lilliefors and anderson-darling tests. *J. Stat. Model. Anal.* **2**, 21–33 (2011).
52. Steinskog, D. J., Tjøstheim, D. B. & Kvamstø, N. G. A cautionary note on the use of the Kolmogorov–Smirnov test for normality. *Mon. Weather Rev.* **135**, 1151–1157 (2007).
53. Blanca, M. J., Alarcón, R., Arnau, J., Bono, R. & Bendayan, R. Non-normal data: Is ANOVA still a valid option?. *Psicothema* **29**, 552–557 (2017).
54. Schmider, E., Ziegler, M., Danay, E., Beyer, L. & Bühner, M. Is it really robust?. *Methodology* **6**, 147 (2010).
55. Salkind, N. J. *Encyclopedia of Research Design* (Sage, 2010).
56. van Doorn, J. *et al.* The JASP guidelines for conducting and reporting a Bayesian analysis. *Psychon. Bull. Rev* **28**, 813–826 (2021).
57. Turi, Z. *et al.* Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation. *Restor. Neurol. Neurosci.* **31**, 275–285 (2013).

58. Yavari, F., Jamil, A., Mosayebi Samani, M., Vidor, L. P. & Nitsche, M. A. Basic and functional effects of transcranial Electrical Stimulation (tES)-An introduction. *Neurosci. Biobehav. Rev.* **85**, 81–92 (2018).
59. Antal, A. & Paulus, W. Transcranial alternating current stimulation (tACS). *Front. Hum. Neurosci.* **7**, 317 (2013).
60. Thut, G., Schyns, P. G. & Gross, J. Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. *Front. Psychol.* **2**, 170 (2011).
61. International Commission on Non-Ionizing Radiation Protection. Guidelines for limiting exposure to time-varying electric and magnetic fields (1 Hz to 100 kHz). *Health Phys.* **99**, 818–836 (2010).
62. Bikson, M. & Rahman, A. Origins of specificity during tDCS: anatomical, activity-selective, and input-bias mechanisms. *Front. Hum. Neurosci.* **7**, 688 (2013).
63. Fertonani, A. & Miniussi, C. Transcranial electrical stimulation: what we know and do not know about mechanisms. *Neuroscientist* **23**, 109–123 (2017).
64. Ruhnau, P., Rufener, K. S., Heinze, H.-J. & Zaehle, T. Sailing in a sea of disbelief: in vivo measurements of transcranial electric stimulation in human subcortical structures. *Brain Stimul.* **11**, 241–243 (2018).
65. Delbeke, J., Oozeer, M. & Veraart, C. Position, size and luminosity of phosphenes generated by direct optic nerve stimulation. *Vision. Res.* **43**, 1091–1102 (2003).
66. Chakraborty, D., Truong, D. Q., Bikson, M. & Kaphzan, H. Neuromodulation of axon terminals. *Cereb. Cortex* **28**, 2786–2794 (2018).

Acknowledgements

We thank all members of the Zaehle Lab for their contributions to discussions and their help with piloting the paradigm. We further thank two anonymous reviewers for their helpful comments on earlier versions of this manuscript

Author contributions

C.T., T.Z. and P.R. planned the study. C.T. and P.R. collected and analysed the data and prepared the artwork. All authors participated in interpreting the results, writing the manuscript and approved the final version.

Funding

Open Access funding enabled and organized by Projekt DEAL. This study was supported by the federal state of Saxony-Anhalt and the European Regional Development Fund (ERDF) in the Center for Behavioral Brain Sciences (CBBS, ZS/2016/04/78113) and the Deutsche Forschungsgemeinschaft (DFG; ZA 626/2–1).

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-01482-1

Correspondence and requests for materials should be addressed to C.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 31 October 2023 | Revised: 14 May 2024 | Accepted: 1 July 2024

DOI: 10.1111/psyp.14651

PSYCHOPHYSIOLOGY SPR WILEY

ORIGINAL ARTICLE

Transcranial temporal interference stimulation (tTIS) influences event-related alpha activity during mental rotation

Carsten Thiele[1,2] | **Katharina S. Rufener**[2,3] | **Stefan Repplinger**[1,2] | **Tino Zaehle**[1,2] | **Philipp Ruhnau**[2,4]

[1]Department of Neurology, Otto-von-Guericke-University, University Clinic of Magdeburg, Magdeburg, Germany

[2]Center for Behavioral Brain Sciences (CBBS), Otto-von-Guericke-Universität Magdeburg, Magdeburg, Germany

[3]Department of Psychiatry, Psychotherapy and Psychosomatic Medicine of Childhood and Adolescents, Otto-von-Guericke-University, University Clinic of Magdeburg, Magdeburg, Germany

[4]School of Psychology and Humanities, University of Central Lancashire, Preston, UK

Correspondence
Carsten Thiele, Medizinische Fakultät, Universitätsklinikum Magdeburg A.ö.R., Leipziger Str. 44, Magdeburg 39120, Germany.
Email: carsten.thiele@med.ovgu.de

Philipp Ruhnau, School of Psychology and Humanities, University of Central Lancashire, Preston PR1 2HE, UK.
Email: pruhnau@uclan.ac.uk

Funding information
Center for Behavioral Brain Sciences (CBBS), Grant/Award Number: ZS/2016/04/78113

Abstract

Non-invasive brain stimulation techniques offer therapeutic potential for neurological and psychiatric disorders. However, current methods are often limited in their stimulation depth. The novel transcranial temporal interference stimulation (tTIS) aims to overcome this limitation by non-invasively targeting deeper brain regions. In this study, we aimed to evaluate the efficacy of tTIS in modulating alpha activity during a mental rotation task. The effects of tTIS were compared with transcranial alternating current stimulation (tACS) and a sham control. Participants were randomly assigned to a tTIS, tACS, or sham group. They performed alternating blocks of resting and mental rotation tasks before, during, and after stimulation. During the stimulation blocks, participants received 20 min of stimulation adjusted to their individual alpha frequency (IAF). We assessed shifts in resting state alpha power, event-related desynchronization (ERD) of alpha activity during mental rotation, as well as resulting improvements in behavioral performance. Our results indicate tTIS and tACS to be effective in modulating cortical alpha activity during mental rotation, leading to an increase in ERD from pre- to poststimulation as well as compared to sham stimulation. However, this increase in ERD was not correlated with enhanced mental rotation performance, and resting state alpha power remained unchanged. Our findings underscore the complex nature of tTIS and tACS efficacy, indicating that stimulation effects are more observable during active cognitive tasks, while their impacts are less pronounced on resting neuronal systems.

KEYWORDS

event-related desynchronization, mental rotation, non-invasive brain stimulation, temporal interference stimulation, transcranial alternating current stimulation

1 | INTRODUCTION

Transcranial electrical stimulation (tES) is being researched as a promising therapeutic intervention for a diverse range of neurological and psychiatric disorders (Cho et al., 2022; Yang et al., 2021), including depression (Alexander et al., 2019; Brunoni et al., 2013; Wang et al., 2022), anxiety (Stein et al., 2020), dyslexia (Marchesotti et al., 2020; Rufener & Zaehle, 2021), schizophrenia (Brunelin et al., 2022; Pinault, 2017), attention deficit hyperactivity disorder (ADHD) (Salehinejad et al., 2019, 2020), Alzheimer's disease (Pini et al., 2022; Rajji, 2019), and stroke rehabilitation (Khan et al., 2022; Solomons & Shanmugasundaram, 2019). This non-invasive method involves the application of weak electric currents through electrodes attached to the scalp, modulating neuronal activity in underlying cortical regions, leading to both behavioral and electrophysiological changes (Antal et al., 2014; Guleyupoglu et al., 2013; Khadka & Bikson, 2023; Paulus et al., 2016; Vosskuhl et al., 2018; Zaghi et al., 2010).

One variant of tES, transcranial alternating current stimulation (tACS), applies weak sinusoidal currents to interact with endogenous brain oscillations via neural entrainment (Abd Hamid et al., 2015; Herrmann et al., 2016; Herrmann & Strüber, 2017). Entrainment refers to the synchronization of endogenous oscillations to the externally applied tACS signal, modulating ongoing rhythmic brain activity (Haegens et al., 2011; Reato et al., 2013; Thut et al., 2011). This interaction induces neuronal changes during stimulation (online effects) and persists post-stimulation (offline effects) (Kasten et al., 2016; Veniero et al., 2015; Zaehle et al., 2010). Studies attribute these offline effects to changes in spike-timing-dependent plasticity (STDP) (Vossen et al., 2015, see Vogeti et al., 2022 for a discussion). This neuronal modulation enables tACS to affect a range of cognitive processes, such as working memory (Grover et al., 2022; Hoy et al., 2015; Pahor & Jaušovec, 2018; Reinhart & Nguyen, 2019), attention (Schuhmann et al., 2019), motor function (Wischnewski, Engelhardt, et al., 2019; Wischnewski, Schutter, & Nitsche, 2019), and speech perception (Rufener et al., 2016; Zoefel et al., 2018).

Despite the effectiveness of tACS at the cortical level, a significant limitation is its shallow stimulation depth. The electrical currents remain primarily superficial and quickly diminish in intensity when reaching deeper regions of the brain (Miranda et al., 2013; Opitz et al., 2016). Consequently, in the treatment of clinical conditions such as Parkinson's disease that arise from dysfunctions in deeper brain areas (Herrington et al., 2016; Limousin & Foltynie, 2019; Volkmann, 2004), invasively implanted electrodes remain the most viable approach for deep brain stimulation.

However, recent findings highlight the potential for a new method, which may be capable of non-invasive deep brain stimulation. Grossman et al. (2017) successfully demonstrated the feasibility of transcranial temporal interference stimulation (tTIS), showing that it could induce motor activity in rodents by stimulating cortical areas. This method simultaneously applies two high-frequency (≥1 kHz) sinusoidal electric fields $\bar{E}_1$ and $\bar{E}_2$ with a frequency offset Δf to the brain. The superposition of both fields creates an interference which results in a low-frequency amplitude-modulated waveform at Δf, which is at the "envelope" or "beat" frequency (e.g., $\Delta f = 10$ Hz, generated via $f_1 = 1000$ Hz, $f_2 = f_1 + \Delta f = 1010$ Hz). This envelope frequency is within the receptive frequency range of neurons and leads to entrainment of endogenous oscillations, causing neuronal modulation (Cao et al., 2020; Conta et al., 2022; Esmaeilpour et al., 2021; Mirzakhalili et al., 2020; Rampersad et al., 2019; Wang et al., 2023). A key aspect of tTIS is that due to an inherent low-pass property, neurons are unable to entrain to oscillations ≥1 kHz (Hutcheon & Yarom, 2000). TTIS leverages this by intentionally setting its carrier frequency f_1 above this threshold to limit its entrainment effect only to the low-frequency amplitude-modulated waveform. A further advantage of tTIS is its high focality (compared to tACS), as the locus of stimulation is limited to the area of maximum interference (i.e., where both electric fields have the same intensity). The intensity of the amplitude-modulated waveform is strongest only within this specific area, minimizing co-stimulation of adjacent areas and thereby enhancing the precision of the stimulation (Conta et al., 2021; Khatoun et al., 2021; Terasawa et al., 2022). Simulations and modeling approaches allow for the guidance of this interference point toward any region in the brain, including subcortical areas (Song 2019; Lee, 2021; Stoupis and Samaras 2022; Terasawa et al., 2022; Conta et al., 2021). The enhanced penetration depth is further facilitated by the higher conductance values of tissues for electric currents in the kHz range, enabling the currents to reach deeper regions (Gabriel et al., 1996). Therefore, tTIS could lay the foundation for non-invasive deep brain stimulation, potentially enabling the treatment of neurological deficits that currently require invasive procedures like deep brain stimulation (DBS) (Grossman et al., 2018).

A comparable method of electrostimulation was proposed half a century ago and tested for its capabilities in electro-anesthesia (Brown, 1975; Sachkov et al., 1967). While this method dubbed as "electrical interferential current therapy" was later used as a means to stimulate and treat muscle tissues (Goats 1990; Beatti et al.

2011), Grossman et al. (2017) were among the first to demonstrate its efficacy in cortical stimulation as well. Computational modeling studies deliver promising results, suggesting that tTIS can indeed induce neuronal entrainment (Karimi et al., 2019; Lee, 2021; Su et al., 2021; Zhu et al., 2022). But the body of in vivo research on tTIS in humans remains sparse and presents conflicting findings. Among the few studies, Ma et al. (2021) demonstrated improved motor functions after tTIS, while Zhu et al. (2022) observed an increased functional connectivity in the motor cortex. However, other studies using magnetoencephalography (MEG) (Conta et al., 2022) or electroencephalography (EEG) (Iszak et al., 2023) found no effect of tTIS on posterior α-power, underscoring the need for further investigation. Consequently, our aim in this study was to gather empirical evidence on the stimulation effect of tTIS in an in vivo experiment in humans to provide a proof-of-concept.

In a recent study, we investigated the efficacy of amplitude-modulated waveforms, as used in tTIS, to cause neuronal activation. For this, we used induced retinal phosphenes as an indicator for stimulation efficacy (Thiele et al., 2021). Our results revealed that unlike tACS, amplitude-modulated waveforms failed to produce phosphenes even at intensities significantly higher than those required for tACS, which was also replicated in Iszak et al. (2023). These findings align with existing literature suggesting that amplitude-modulated waveforms necessitate greater stimulation intensities for effective neuronal activation compared to sinusoidal alternating currents, as used in tACS (Esmaeilpour et al., 2021; Rampersad et al., 2019). We concluded that although effects in the form of neuronal *activation* may not be achievable, the potential for neuronal *modulation* remains. Therefore, in the present study, we evaluated the efficacy of tTIS in humans by analyzing its capability for causing neuronal modulations.

For this, we induced and measured changes in cortical alpha activity after stimulation with tTIS. Studies on alpha oscillations generally refer many cognitive functions to alpha activity, including perception (Romei et al., 2012), intelligence (Doppelmayr et al., 2002), or top-down control (Sherman et al., 2016). The prevailing understanding of the role of alpha activity in cognition is that it directs the flow of information by gating task-irrelevant or distracting information, thereby allowing for more focused attention on task-relevant information (Foxe & Snyder, 2011; Klimesch et al., 2011). Consequently, an increase in alpha activity within a region is observed when there is active suppression of interfering information or processes. Conversely, a decrease in alpha activity is thought to facilitate information processing and cognitive performance (Foxe & Snyder, 2011; Haegens et al., 2011; Jensen & Mazaheri, 2010; Rihs et al., 2009; Sauseng et al., 2005; Zumer et al., 2014). TACS studies can use this link to their advantage by modulating alpha activity to enhance information processing (Schutter & Wischnewski, 2016). Studies have validated this concept using Shepard's mental rotation task (Shepard & Metzler, 1971), with tACS in the alpha range significantly improving task performance compared to sham stimulation (Kasten et al., 2018; Kasten & Herrmann, 2017). Building on these findings, the objective of this study was to explore whether tTIS could produce a similar effect. Specifically, we aimed to increase alpha power using tTIS, with the goal of enhancing performance in a mental rotation task. To differentiate between true stimulation effects and effects due to time-on-task, we included a sham stimulation group as a control. Additionally, a tACS group was included as a benchmark of stimulation effects using this paradigm (Kasten et al., 2018; Kasten & Herrmann, 2017) allowing for a direct comparison of the stimulation efficacy of tTIS and tACS.

We quantified the stimulation effect by calculating event-related spectral perturbation (ERSP) (Makeig et al., 2004; Pfurtscheller & Aranibar, 1977; Pfurtscheller & Da Silva, 1999) in the alpha band (8–13 Hz). ERSP measures the change in spectral power, or oscillatory activity, triggered by an event, like the presentation of a visual stimulus. This measurement distinguishes between two phenomena: an increase in spectral power, known as event-related synchronization (ERS); and a decrease in spectral power, referred to as event-related desynchronization (ERD). Klimesch et al. (2007) argue that an ERD in the alpha band is associated with a release of inhibitory processes, allowing for subsequent neuronal activation and facilitation of information processing (see also Pfurtscheller, 1997). This hypothesis has been supported by studies using transcranial magnetic stimulation (TMS) (Klimesch et al., 2003) and more recently, by studies using tACS to increase ERD and improve performance in a mental rotation task (Kasten et al., 2018; Kasten & Herrmann, 2017). However, conversely, some studies not using brain stimulation techniques have instead reported improved performance with reduced ERD, postulating a neural efficiency hypothesis, which suggests that skilled individuals use fewer brain resources during task performance (Chen et al., 2013; Riečanský & Katina, 2010). Given our use of brain stimulation techniques, we hypothesize our results to be in line with those found in the studies of Kasten et al. (2018; 2017), expecting an ERD increase in tTIS and tACS groups compared to the sham group, as well as a corresponding improvement in mental rotation performance.

Numerous studies have demonstrated the neuronal modulation effect of tACS and its associated increase in alpha power (Berger et al., 2018; Kasten et al., 2016; Veniero et al., 2015; Zaehle et al., 2010). Considering that

a mental rotation task naturally leads to significant alpha modulations by suppressing alpha activity during task execution, this could potentially disrupt the stimulation's entrainment effect. To measure the stimulation effect without the confounding influence of a complex cognitive task, we additionally included resting blocks utilizing a simple vigilance task, a setup also utilized by other studies (Conta et al., 2022; Kasten et al., 2018; Kasten & Herrmann, 2017; Zaehle et al., 2010). We hypothesized a significantly stronger increase in resting state alpha power from pre- to poststimulation in the tTIS and tACS groups compared to the sham group.

2 | METHODS

2.1 | Participants

Given that no studies on the effects of tTIS on mental rotation have been conducted, we could only approximate the required sample size. We based our estimate on a previous study by Kasten and Herrmann (2017), which reported an effect size of $\eta^2 = 0.27$ for two groups (stimulation vs. sham). Using these parameters, we conducted a power analysis with G*Power (Ver. 3.1.9.7, Faul et al., 2007), setting an alpha error probability of 0.05 and a power of 0.95 for a *t*test between two independent means. This analysis suggested a required sample size of 16 participants per group. While we recognize that this is only an approximate solution, it provided a reasonable starting point for our research. In total, we recruited 67 participants who were randomly assigned to either the tTIS, tACS, or sham group. Of the 67 participants, 48 were eligible for data analysis. This was, on the one hand, due to technical issues (a coding error in the Matlab stimulation function) leading to the exclusion of nine individuals from the data analysis. Additionally, 10 participants were excluded due to the absence of a discernible peak in the alpha band (8–13 Hz) during the first block of the resting task, which was crucial to extract the individual alpha frequency (IAF) (see Electrical Stimulation) for subsequent stimulation. As a result, our analysis included data from 48 participants, divided into three stimulation conditions: tACS ($n = 18$; 11 female, 7 male; mean age = 23.7, *SD* age = 4.52), tTIS ($n = 16$; 10 female, 6 male; mean age = 21.8, *SD* age = 2.46), and sham ($n = 14$; 11 female, 3 male; mean age = 22.9, *SD* age = 2.85).

Eligible participants for this study had to meet the following criteria: no history of epileptic seizures or psychiatric or neurological disorders, no metal or electric implants in their body, free of medication affecting the central nervous system, non-smoking, and normal or corrected-to-normal vision. Before the experiment, participants were informed about the experimental procedure and the potential adverse effects of electrostimulation and were required to give written informed consent. This study was approved by the local ethics committee of the University Clinic of Magdeburg and conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2 | EEG

EEG data were acquired using passive Ag-AgCl electrodes (EasyCap, Brain Products, Gilching, Germany) positioned at Fz, Pz, P7, and P8 following the international 10–20 system. The reference electrode was placed on the tip of the participant's nose, while the ground electrode was positioned at AFz. In addition, electrodes were placed vertically (VEOG) and horizontally (HEOG) to the right eye and referenced to the nose electrode, to control for eye movements and eyeblinks. To increase electrode to skin conductivity, we applied a conductive paste (Abralyt 2000 abrasive electrolyte-gel, Brain Products, Gilching, Germany), ensuring impedances remained below 5 kΩ. Data were sampled at a rate of 1000 Hz using a BrainAmp DC amplifier (Brain Products, Gilching, Germany).

2.3 | Electrical stimulation

We used one (or two in case of TI) battery-operated stimulator system (DC-Stimulator Plus with extended frequency range for TI, NeuroConn GmbH, Ilmenau, Germany) connected to a PC via a digital-to-analog converter (NI USB-6212, National Instruments, Austin, TX, USA) to generate and send the stimulation signal through a custom Matlab (version 2020a, Mathworks, Natick, USA) script [Correction added on August 1, 2024, after first online publication: The manufacturer name has been updated in the previous sentence.]. The stimulation was administered using surface conductive rubber electrodes (NeuroConn GmbH, Ilmenau, Germany) attached to the subject's head using an adhesive, electrically conductive paste (Ten20, D.O. Weaver, Aurora, CO, USA). Electrode impedances were kept below 5 kΩ to ensure optimal conductivity.

As studies suggest that stimulation in the alpha band is much more effective if the stimulation frequency matches the participants' IAF (Huang et al., 2021; Kasten et al., 2019; Schutter & Wischnewski, 2016), we determined the IAF based on the EEG data recorded in the first resting block at EEG electrode Pz and used it as the stimulation frequency.

For the tACS and sham groups, we used a parieto-occipital electrode montage consisting of a 5×7 cm electrode placed over Cz and a 4×4 cm electrode placed over Oz, with a stimulation intensity of 1 mA peak-to-peak. This montage has shown to increase power in the alpha range in posterior areas (Kasten et al., 2019) and to improve performance in a mental rotation task (Kasten et al., 2018; Kasten & Herrmann, 2017). Using SimNIBS 3.0 (Thielscher et al., 2015), we simulated the electric field of this montage to confirm sufficient electric field strengths (>0.2 V/m) were being delivered to the targeted posterior brain areas (see Figure 1c Left). Participants in the tACS group received 20 min of stimulation in total, split between the $\text{Rest}_{\text{Stimulation}}$ and $\text{Rotation}_{\text{Stimulation}}$ blocks, whereas those in the sham group received stimulation for only 30 s at the beginning of a stimulation block. Stimulation intensities were ramped up and down for 10 s at the beginning and end, respectively.

For tTIS, we used a custom SimNIBS script based on the formula proposed by Grossman et al. (2017) to determine a tTIS montage targeting approximately the same posterior regions as our tACS montage. Our simulations indicated that two pairs of two round electrodes (34 mm diameter) each allow for stimulation of posterior brain areas (see Figure 1c Right). The first pair of electrodes was positioned with one electrode on P4 and the other between I1 and O1. The second pair was arranged with one electrode on P3 and the other placed between I2 and O2. Since tTIS requires a higher stimulation intensity to achieve a comparable stimulation efficacy as tACS (Esmaeilpour et al., 2021), we used a stimulation intensity of 2 mA peak-to-peak for stimulation with tTIS. This is still a safe (Cassarà et al., 2022; Piao et al., 2022) and tolerable stimulation intensity, as tTIS uses high-frequency stimulation, to which somatosensory perception is less sensitive (Fertonani et al., 2015; Hsu et al., 2021; Zeng et al., 2019) and thus can be tolerated by participants without adverse effects. Anecdotally, many participants in our study reported not feeling tTIS at all. We selected a carrier frequency of 1 kHz balancing the need to maximize stimulation efficacy and minimizing unintended stimulation effects related to the carrier frequency (Esmaeilpour et al., 2021; Grossman et al., 2017; Rampersad et al., 2019). For the envelope frequency, we used the IAF. Using tTIS, the envelope is determined by the difference in stimulation frequencies of E_1 and E_2. This resulted in stimulation frequencies of $f_1 = 1000$ Hz and $f_2 = 1000$ Hz + IAF. As was the case for tACS, the stimulation duration for tTIS was 20 min in total, split between the $\text{Rest}_{\text{Stimulation}}$ and $\text{Rotation}_{\text{Stimulation}}$ blocks, with the stimulation being ramped up at the beginning and down at the end over a period of 10 s.

2.4 | Data analysis

The acquired electrophysiological data were processed and analyzed using Matlab 2020a (The MathWorks Inc., Natick, MA, USA) in conjunction with the Fieldtrip toolbox (Oostenveld et al., 2011). Statistical analysis was performed using JASP version 0.17.1 (JASP Team, 2023) and Jamovi version 2.3 (The Jamovi project 2022).

To assess behavioral performance in the mental rotation task, measures of task accuracy, that is, correct answers and reaction time (RT), were analyzed. One-way analyses of variance (ANOVAs) were conducted on accuracy and RT of the $\text{Rotation}_{\text{Baseline}}$ Block, with the between-subject factor *Stimulation* (tTIS, tACS, sham) to identify potential baseline differences. Subsequently, group differences in task performance before, during, and after stimulation were examined using repeated measures ANOVAs (rmANOVAs). The rmANOVAs included the within-subject factor *Block* ($\text{Rotation}_{\text{Baseline}}$, $\text{Rotation}_{\text{Stimulation}}$, $\text{Rotation}_{\text{Poststim}}$) and the between-subject factor *Stimulation* (tTIS, tACS, sham). Further, to analyze the effects of stimulation on behavioral performance and adjust for individual baseline differences, we've calculated change measures ΔAccuracy (Accuracy in $\text{Rotation}_{\text{Poststim}}$ − Accuracy in $\text{Rotation}_{\text{Baseline}}$) and ΔRT (RT in $\text{Rotation}_{\text{Poststim}}$ − RT in $\text{Rotation}_{\text{Baseline}}$). This shows whether performance increased or decreased, comparing the pre- and poststimulation performance, to help us understand changes due to stimulation, regardless of initial performance levels. Change measures, ΔAccuracy and ΔRT, were then analyzed using separate ANOVAs using between-subject factor *Stimulation* (tTIS, tACS, sham).

In our ANOVAs, we report both partial eta squared (η_p^2), which shows the variance explained in our model but may overestimate effects, and generalized eta squared (η_G^2), which offers consistent effect sizes across studies but is less specific to our design. This approach balances detailed insights with broader comparability.

For EEG analysis of alpha activity, we focused on electrode Pz for the analysis similar to previous studies (Kasten et al., 2016; Kasten & Herrmann, 2017; Zaehle et al., 2010). To analyze changes in resting state alpha activity, data of all stimulation-free resting blocks ($\text{Rest}_{\text{Baseline}}$, $\text{Rest}_{\text{Poststim 1}}$, $\text{Rest}_{\text{Poststim 2}}$, $\text{Rest}_{\text{Poststim 3}}$) were analyzed. EEG data collected during the $\text{Rest}_{\text{Stimulation}}$ block could not be analyzed due to significant stimulation artifacts. Blocks were epoched into 1-second non-overlapping segments. Segments containing major artifacts (e.g., muscle artifacts) were removed after visual inspection via fieldtrips ft_rejectvisual function. Afterward, power in the IAF band (IAF +/−1 Hz) was calculated by performing fast Fourier transforms (FFTs)

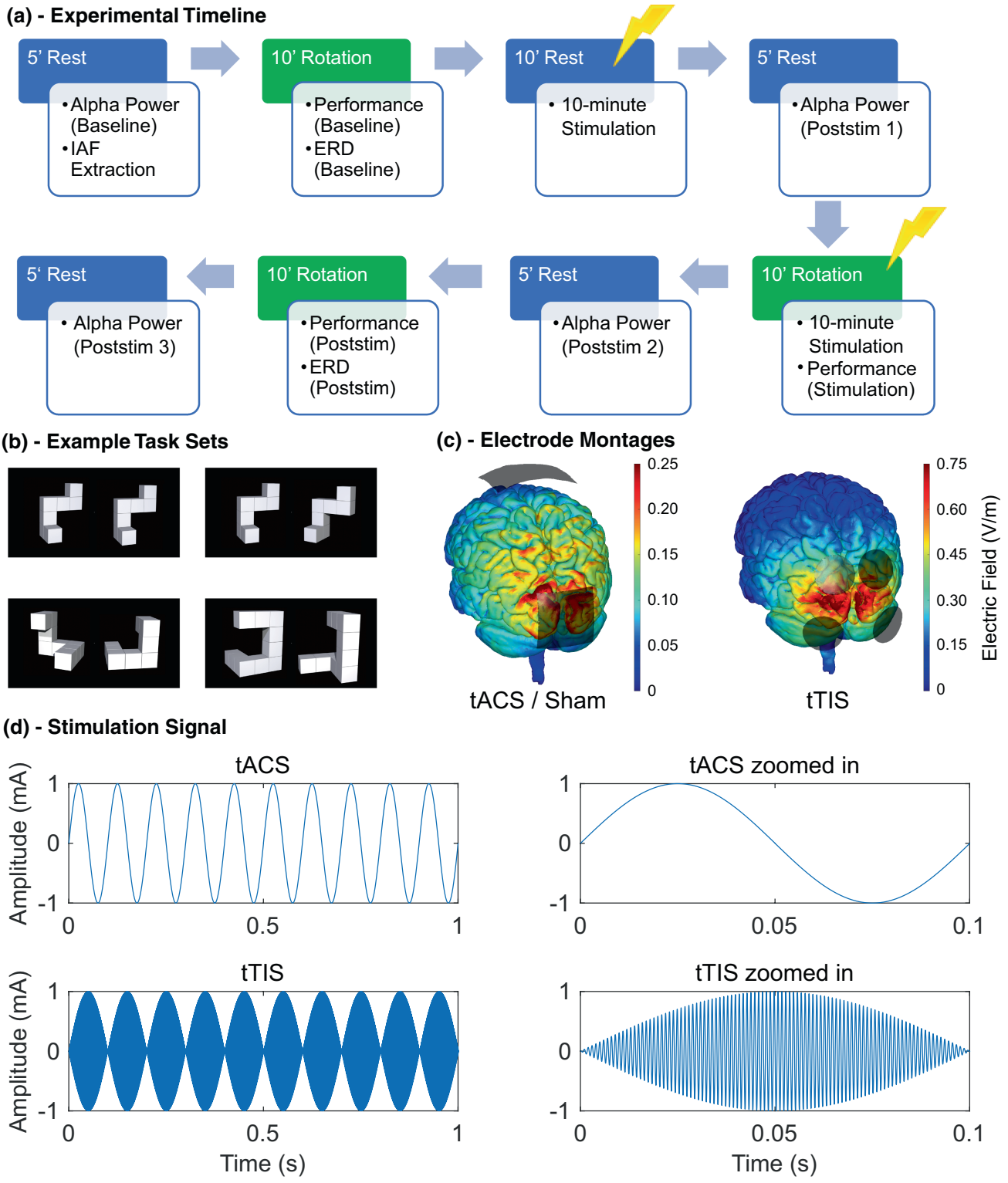

FIGURE 1 Experimental design. (a) Experimental Timeline. Chronological sequence of events during the experiment. Participants began with a resting block, followed by alternating blocks of mental rotation and resting blocks. Stimulation was administered during the second resting block and the second mental rotation block (indicated with a lightning symbol). (b) Example Task Set. Illustration of example task sets used in the study. Geometric figures, based on Shepard's mental rotation task, were presented to participants. They were required to determine whether the figures were mirrored or not. (c) Electrode Montages. Depending on the (random) group assignment, participants received either tACS, sham, or tTIS. (d) Stimulation Signal Exemplified. The figure demonstrates the stimulation signals used in the study. On the left side, 10 Hz tACS sine waves are compared to a tTIS amplitude-modulated waveform with a 10 Hz envelope frequency. The right side provides a zoomed-in view of the waveforms, highlighting the high-frequency carrier component of tTIS.

using a Hanning window and 2-second zero padding. In addition, using the *fitting oscillations & one-over f* (FOOOF) method (Donoghue et al., 2020), data were split into periodic and aperiodic components to distinguish rhythmic activity from concurrent power-spectral 1/f modulations. For data analysis, only periodic components were analyzed, as these reflect frequency-specific rhythmic activity and are free of power changes affecting all frequency bands, which can be caused, for instance, by technical issues such as changes in electrode impedances. The periodic component in the IAF Band was then analyzed using an rmANOVA with the within-subject factor *Block* ($\text{Rest}_{\text{Baseline}}$, $\text{Rest}_{\text{Poststim 1}}$, $\text{Rest}_{\text{Poststim 2}}$, $\text{Rest}_{\text{Poststim 3}}$) and between-subject factor *Stimulation* (tTIS, tACS, sham) to detect changes in resting state alpha power. Additionally, we calculated a change measure, $\Delta\text{Alpha}_{\text{Rest}}$ (=Alpha power in $\text{Rest}_{\text{Poststim3}}$ − Alpha power in $\text{Rest}_{\text{Baseline}}$), and conducted an ANOVA with the between-subject factor *Stimulation* (tTIS, tACS, sham). This analysis focused specifically on increases in alpha activity, independent of individual alpha levels.

To capture changes in the IAF-band during the mental rotation task, ERSP was calculated for baseline and poststim mental rotation blocks. Again, data acquired during the stimulation block could not be analyzed due to the stimulation artifact. Data in the mental rotation blocks were segmented into 10-second epochs, spanning from 3 s before to 7 s after stimulus presentation. Epochs containing major artifacts were rejected after visual inspection using fieldtrips *ft_rejectvisual* function. Remaining epochs were analyzed using FFTs with a Hanning-tapered sliding window with a fixed length of 1 s moving in steps of 50 ms along each segment and 2-second zero padding (as in Kasten & Herrmann, 2017). Afterward, ERSP values were calculated as:

$$\text{ERSP}_t = \frac{R_t - A_t}{R_t} \times 100$$

In this formula, R is defined as the power during a reference period, specifically before the stimulus is presented. In our study, R was the power in the IAF band (IAF +/− 1 Hz) calculated from −2 to 0 s relative to stimulus onset. On the other hand, A is defined as the power of the frequency of interest during a test period, which is after the stimulus presentation. For A, we defined it as the power in the IAF band from 0 to 3 s following stimulus onset. Furthermore, t is defined as the trial number, as we calculated the ERSP on a trial-by-trial basis, based on prior studies (Kasten & Herrmann, 2017; Nakayashiki et al., 2014). To acquire an average ERSP for a block, ERSPs of all trials were averaged in each block for each subject. Resulting positive values indicate an ERD, reflecting a drop in power in the frequency band of interest at stimulus onset, whereas negative values indicate an ERS, reflecting an increase in power at stimulus onset. Based on previous work (Kasten et al., 2018; Kasten & Herrmann, 2017), we expected to observe an ERD. To explore potential differences in baseline ERD between stimulation groups, the ERD in the baseline block was analyzed using an ANOVA with between-subject factor *Stimulation* (tTIS, tACS, sham). ERD values were then fed into an rmANOVA with within-subject factor *Block* ($\text{Rotation}_{\text{Baseline}}$, $\text{Rotation}_{\text{Poststim}}$) and between-subject factor *Stimulation* (tTIS, tACS, sham). Again, a change measure ΔERD (ERD in $\text{Rotation}_{\text{Poststim}}$ − ERD in $\text{Rotation}_{\text{Baseline}}$) was calculated and subjected to an ANOVA with between-subject factor *Stimulation* (tTIS, tACS, sham) to titrate changes in ERD from pre- to poststimulation.

Finally, we investigated if changes in task performance were correlated with changes in ERD values. To achieve this, we correlated ΔERD with ΔAccuracy and ΔRT. Prior to further analysis, we confirmed that all variables exhibited normal distribution based on the Shapiro–Wilk test (ΔERD: $W = 0.985$, $p = .796$; ΔAccuracy: $W = 0.972$, $p = .31$; ΔRT: $W = 0.973$, $p = .337$). Subsequently, we performed Pearson's correlational analysis to examine the potential influence of changes in ERD on changes in task performance measures.

For rmANOVA results we report partial eta squared (η_p^2) to focus on effect size within our chosen design, as well as generalized eta squared (η_G^2) to facilitate comparing effect sizes across studies.

3 | RESULTS

3.1 | Experimental design

The experimental tasks were displayed on a Samsung SyncMaster SA450 placed at a distance of approximately 100 cm from the participants. For the experimental presentation, we utilized Psychtoolbox 3 (Kleiner et al., 2007) which was implemented using Matlab 2020a (The MathWorks Inc., Natick, MA, USA).

During the experiment, participants engaged in alternating resting blocks and mental rotation blocks (see Figure 1a). During the resting blocks ($\text{Rest}_{\text{Baseline}}$, $\text{Rest}_{\text{Stimulation}}$, $\text{Rest}_{\text{Poststim 1}}$, $\text{Rest}_{\text{Poststim 2}}$, $\text{Rest}_{\text{Poststim 3}}$), participants engaged in a simple vigilance task as employed by previous studies (Kasten & Herrmann, 2017; Zaehle et al., 2010). In this task, participants were shown a fixation cross at the center of the screen, which could rotate

by 45 degrees for a duration of 500 ms. The onset of the rotation varied, occurring randomly between 30 and 40 s. Upon noticing the rotation, participants were required to press a response button within a 2-second window following the stimulus onset. This task served a dual purpose: maintaining the subjects' attention and facilitating the collection of clean EEG data. Resting blocks consisted of a 4-minute vigilance task followed by a 1-minute break. The exception was the $Rest_{Stimulation}$ block, whose duration was doubled to 10 min to accommodate the necessary 10-minute stimulation period.

In mental rotation blocks ($Rotation_{Baseline}$, $Rotation_{Stimulation}$, $Rotation_{Poststim}$), participants performed a Shepard's mental rotation task (Shepard & Metzler, 1971) which involved presenting two geometrical figures (see Figure 1b) that could be (1) rotated and/or (2) mirrored in relation to each other. Participants were tasked with mentally rotating the figures to matching angles, in order to determine whether the figures were mirrored or not. The figures could be rotated relative to each other by 0°, 50°, 100°, or 150°, with larger rotation angles increasing the task's difficulty. Participants indicated their responses by pressing the "J" key for mirrored trials and the "n" key for non-mirrored trials. Each rotation block comprised 48 trials, with 12 trials per rotation angle. Half of the trials contained mirrored figures. A trial began with the presentation of a fixation cross in the middle of the screen for 3 s, followed by the presentation of the geometric figures which lasted for 7 s. Participants were instructed to answer as fast and accurately as possible. A mental rotation block consisted of a 4-minute task, followed by a 1-minute break, then another 4-minute task, and a concluding 1-minute break. The stimuli for the task were selected from a published open-source stimulus set (Ganis & Kievit, 2015). During the $Rotation_{Stimulation}$ block, participants received 10 min of stimulation.

At the beginning of the experiment, participants completed a questionnaire to assess exclusion criteria and were informed about the planned experimental procedure and tasks. Following this, the montages for electrical stimulation (see Figure 1c) and EEG were set up. Participants then engaged in the alternating blocks of resting and mental rotation tasks. Depending on the group, electrical stimulation of either tTIS, tACS, or sham (see Figure 1d) was administered in the second resting block and in the second rotation task block (see Figure 1a). At the end of the experiment, participants filled out a questionnaire about possible adverse effects of the stimulation (nausea, headache, sensations of pain/burning/itching/reddening at the stimulated area) (Brunoni et al., 2011). Afterward, participants were debriefed about the aim of this study and reimbursed for their time, either with course credit or monetarily.

3.2 | Resting-state alpha power

Analysis of resting-state alpha power revealed a main effect *Block* [$F(3,135) = 22.27, p < .001, \eta_p^2 = 0.331, \eta_G^2 = 0.051$]. Subsequent post-hoc t tests indicated an increase in alpha power throughout the experiment [$Rest_{Baseline}$ vs. $Rest_{Poststim\ 1}$, $Rest_{Baseline}$ vs. $Rest_{Poststim\ 2}$, $Rest_{Baseline}$ vs. $Rest_{Poststim\ 3}$, $Rest_{Poststim\ 1}$ vs. $Rest_{Poststim\ 2}$, $Rest_{Poststim\ 1}$ vs. $Rest_{Poststim\ 3}$: all $t(45) \geq 3.263, p_{tukey} \geq 0.011$], with the exception of the comparison between blocks $Rest_{Poststim\ 2}$ and $Rest_{Poststim\ 3}$ [$t(45) = 0.671, p_{tukey} = 0.908$] (see Figure 2a). Neither a significant main effect of *Stimulation* on resting state alpha power [$F(2,45) = 1.19, p = .314, \eta_p^2 = 0.050, \eta_G^2 = 0.045$] nor an interaction between *Stimulation* × *Block* [$F(6,135) = 0.931, p = .475, \eta_p^2 = 0.040, \eta_G^2 = 0.004$] were observed. The ANOVA on $\Delta Alpha_{Rest}$ did not reveal a main effect *Stimulation* [$F(2,45) = 1.15, p = .327, \eta_p^2 = 0.048$]. In sum, these findings indicate that alpha power did increase over the course of the experiment, but this increase did not differ between stimulation groups.

3.3 | Mental rotation accuracy

The analysis of baseline accuracy in the $Rotation_{Baseline}$ block did not reveal a statistically significant main effect for *Stimulation* [$F(2,45) = 1.38, p = .263, \eta_p^2 = 0.058$], indicating comparable baseline performance between groups. The rmANOVA across all mental rotation blocks (see Figure 2b) demonstrated a significant main effect *Block* [$F(2,90) = 7.14, p < .001, \eta_p^2 = 0.137, \eta_G^2 = 0.046$]. Post-hoc tests revealed this to be due to a statistically significant improvement in accuracy from $Rotation_{Baseline}$ to $Rotation_{Poststim}$ [$t(45) = 3.77, p_{tukey} = .001$] but no statistically significant difference in accuracy between $Rotation_{Baseline}$ and $Rotation_{Stimulation}$ [$t(45) = 1.96, p_{tukey} = .135$] or between $Rotation_{Stimulation}$ and $Rotation_{Poststim}$ blocks [$t(45) = 1.81, p_{tukey} = .176$]. We found no main effect *Stimulation* on accuracy [$F(2,45) = 1.27, p = .291, \eta_p^2 = 0.053, \eta_G^2 = 0.038$] nor a *Block* × *Stimulation* interaction [$F(4,90) = 1.34, p = .260, \eta_p^2 = 0.056, \eta_G^2 = 0.018$]. For descriptive results of the mental rotation accuracy, see Table 1. The analysis of ΔAccuracy did not reveal a main effect *Stimulation* [$F(2,45) = 0.653, p = .525, \eta_p^2 = 0.028$], indicating that all groups experienced comparable increases in task accuracy.

Notably, accuracy levels in the $Rotation_{Poststim}$ block approached the ceiling across all stimulation groups, offering minimal room for observable improvements attributable to stimulation. This suggests that the lack of significant stimulation effects could be attributed to ceiling effects, limiting the ability to draw conclusions about the impact of stimulation on task accuracy. Collectively, the findings suggest that while accuracy on the mental rotation task

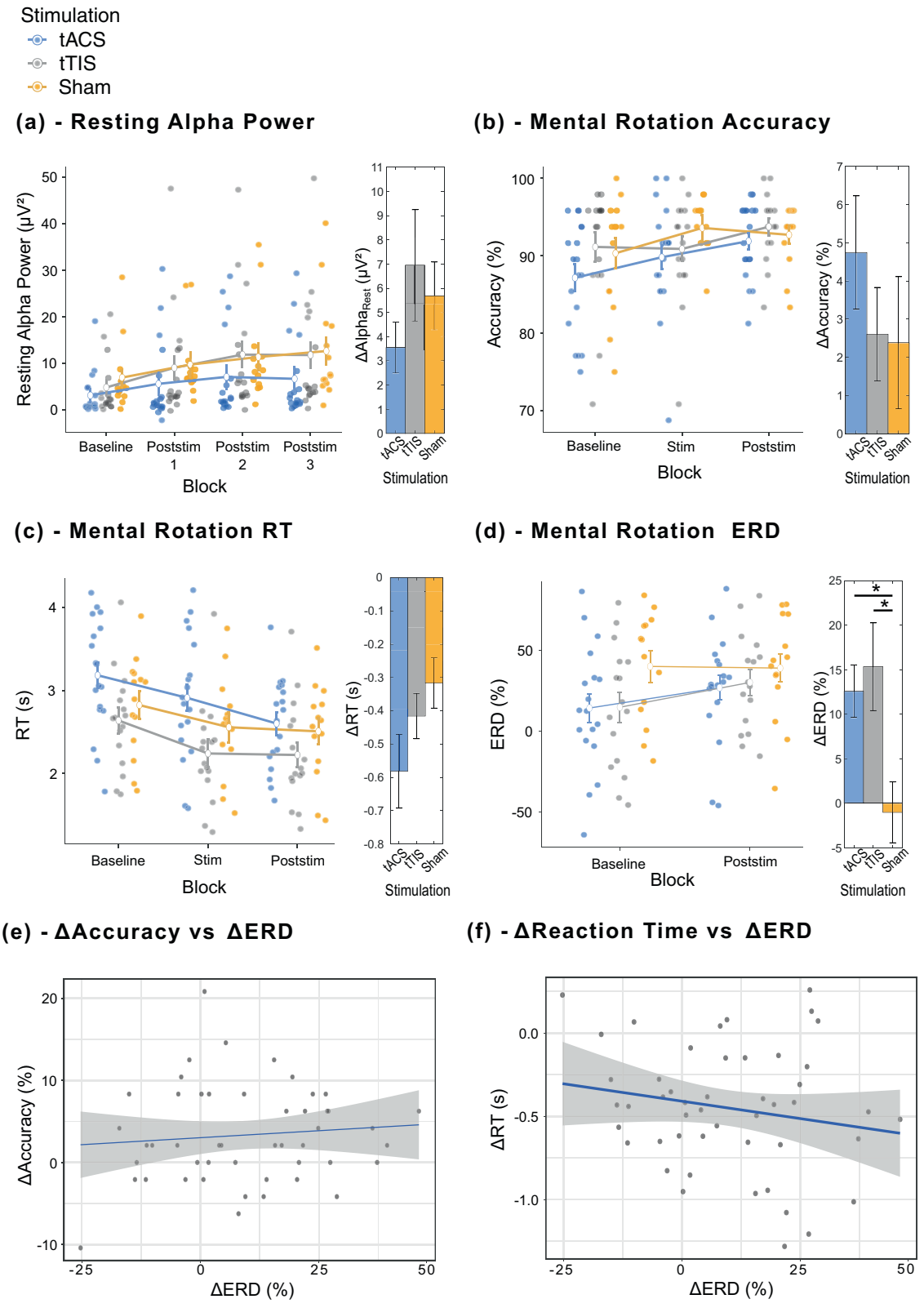

FIGURE 2 Overview of main results. (a) Mental rotation accuracy improves from the Baseline to the Poststim block, with no statistically significant differences observed between stimulation groups. (b) Similarly, reaction time in the mental rotation task shows improvement from the Baseline to the Poststim block. (c) In resting blocks, alpha power increases over the course of the experiment from Baseline to Poststim 2, where alpha activity plateaus and does not further increase in Poststim 3. No significant differences were observed between stimulation groups. (d) In the mental rotation task, ERD values increased from Baseline to Poststim blocks for tTIS and tACS groups, while remaining stable for the sham group. (e, f) Correlation analysis reveals no significant correlation between changes in ERD and changes in task accuracy (e) or RT (f).

TABLE 1 Descriptive statistics of behavioral data during mental rotation.

		Accuracy (%)				RT (s)			
Stimulation	**Block**	**Mean**	**SD**	**Minimum**	**Maximum**	**Mean**	**SD**	**Minimum**	**Maximum**
tTIS	Baseline	91.1	7.70	70.8	97.9	2.64	0.59	1.75	4.06
	Stimulation	90.9	6.97	70.8	100.0	2.24	0.62	1.29	3.92
	Poststim	93.7	4.37	83.3	100.0	2.22	0.62	1.33	3.71
tACS	Baseline	87.2	7.15	75.0	95.8	3.18	0.68	1.78	4.18
	Stimulation	89.8	7.39	68.8	100.0	2.91	0.76	1.58	4.21
	Poststim	91.9	4.89	81.3	97.9	2.60	0.55	1.67	3.76
Sham	Baseline	90.3	7.43	75.0	100.0	2.82	0.60	1.79	3.89
	Stimulation	93.6	3.32	85.4	97.9	2.56	0.61	1.52	3.75
	Poststim	92.7	3.98	83.3	95.8	2.51	0.58	1.43	3.51

Abbreviations: Poststim, poststimulation, *SD* = standard deviation.

TABLE 2 Post-hoc analysis of the significant *Block* × *Stimulation* interaction effect on RT during mental rotation.

Block	**Stimulation**		**Block**	**Stimulation**	**Mean diff.**	***SE***	**df**	***t***	p_{tukey}
Baseline	tTIS	-	Stim	tTIS	0.40	0.08	45	5.27	< .001
Baseline	tTIS	-	Poststim	tTIS	0.42	0.09	45	4.59	0.001
Stim	tTIS	-	Poststim	tTIS	0.02	0.08	45	0.23	1.000
Baseline	tACS	-	Stim	tACS	0.27	0.07	45	3.82	0.011
Baseline	tACS	-	Poststim	tACS	0.58	0.09	45	6.80	< .001
Stim	tACS	-	Poststim	tACS	0.31	0.07	45	4.26	0.003
Baseline	Sham	-	Stim	Sham	0.27	0.08	45	3.31	0.044
Baseline	Sham	-	Poststim	Sham	0.32	0.10	45	3.27	0.048
Stim	Sham	-	Poststim	Sham	0.05	0.08	45	0.60	1.000

Abbreviations: df, degrees of freedom; mean diff., mean difference; Poststim, poststimulation; p_{tukey}, *p*-value with Tukey correction; *SE*, standard error; *t*, *t* value.

significantly increased throughout the experiment, the rate of this improvement did not vary across the different stimulation groups. Therefore, it is most plausible that the observed improvement stems from a training effect rather than the stimulation itself.

3.4 | Mental rotation reaction time

Analysis of baseline differences of RT in the $Rotation_{Baseline}$ block revealed a statistically significant main effect *Stimulation* [$F(2,45) = 3.31$, $p = .046$, $\eta_p^2 = 0.128$]. Post-hoc *t*tests indicated that reaction times in the tACS group were significantly slower than in the tTIS group [$t(45) = 2.52$, $p_{tukey} = .040$], but no differences were found between tTIS and sham [$t(45) = 0.81$, $p_{tukey} = .70$] or tACS and sham groups [$t(45) = 1.60$, $p_{tukey} = .255$]. Since this was RT in the $Rotation_{Baseline}$ block, where no stimulation had yet been administered, the observed differences are likely a random effect due to randomly assigning slower participants to the tACS group, faster subjects to the tTIS group, or a combination of both scenarios.

Comparing RT between all mental rotation blocks (see Figure 2c), we observed a main effect *Block* on RT [$F(2,90) = 45.67$, $p < .001$, $\eta_p^2 = 0.504$, $\eta_G^2 = 0.083$] with post-hoc tests indicating an improvement in RT from $Rotation_{Baseline}$ to $Rotation_{Stimulation}$ [$t(45) = 7.13$, $p_{tukey} < .001$], $Rotation_{Baseline}$ to $Rotation_{Poststim}$ [$t(45) = 8.33$, $p_{tukey} < .001$], and $Rotation_{Stimulation}$ to $Rotation_{Poststim}$ [$t(45) = 2.81$, $p_{tukey} = .020$]. Further, a main effect *Stimulation* was found [$F(2,45) = 3.32$, $p = .045$, $\eta_p^2 = 0.129$, $\eta_G^2 = 0.118$]. Post-hoc analysis revealed this to be due to the tACS group having significantly slower RTs compared to the tTIS group [$t(45) = 2.57$, $p_{tukey} = .035$], which is in line with the found baseline difference between these groups. No difference was found between tTIS and sham [$t(45) = 1.19$, $p_{tukey} = .464$] or tACS and sham groups [$t(45) = 1.26$, $p_{tukey} = .426$]. Additionally, we observed a significant *Block* × *Stimulation* interaction on RT [$F(4,90) = 2.56$, $p = .044$, $\eta_p^2 = 0.102$, $\eta_G^2 = 0.010$].

Post-hoc tests revealed that all stimulation groups exhibited improvements in RT from the $Rotation_{Baseline}$ to the $Rotation_{Stimulation}$ block, as well as from the $Rotation_{Baseline}$ to the $Rotation_{Poststim}$ block (see Table 2 for full post-hoc results). However, the tTIS and sham groups reached a plateau in RT improvement during the $Rotation_{Stimulation}$ block and did not show further improvement from $Rotation_{Stimulation}$ to $Rotation_{Poststim}$. In contrast, the tACS group displayed continued RT improvement even after stimulation, showing further enhancements in the $Rotation_{Poststim}$ block. For descriptive results of the RT, see Table 1. The analysis of ΔRT did not reveal a main effect *Stimulation* [$F(2,45)=2.20$, $p=.123$, $\eta_p^2=0.089$], meaning no differential decrease in RT between stimulation groups could be observed.

Taken together, reaction times significantly improved over the course of the experiment, with RT in the tTIS and sham groups reaching a plateau during stimulation, while the tACS group continued to improve even post-stimulation. It is important to note that the tACS group initially had the slowest RT, thereby having the greatest potential for improvement. This suggests that the difference observed between the tACS group and both the tTIS and sham groups could be attributed to the latter groups reaching an RT ceiling earlier in the $Rotation_{Stimulation}$ block, whereas the tACS group may have required the duration of the $Rotation_{Poststim}$ block to catch up.

3.5 | Mental rotation ERD

The analysis of baseline differences in ERD between stimulation groups did not reveal significant differences [$F(2,45)=2.34$, $p=.108$, $\eta_p^2=0.094$]. The analysis of changes in ERD across mental rotation blocks revealed a main effect *Block* [$F(1,45)=16.15$, $p<.001$, $\eta_p^2=0.264$, $\eta_G^2=0.017$]. This was due to an increase in ERD from the $Rotation_{Baseline}$ to the $Rotation_{Poststim}$ block (see Figure 2d). This indicates a notable shift in task-relevant oscillatory activity. Though there was no statistically significant main effect *Stimulation* on ERD [$F(2,45)=1.41$, $p=.254$, $\eta_p^2=0.059$, $\eta_G^2=0.056$], we observed a significant *Stimulation*×*Block* interaction [$F(2,45)=4.80$, $p=.013$, $\eta_p^2=0.176$, $\eta_G^2=0.010$]. Post-hoc *t*tests revealed this interaction to be driven by an increase in ERD from the $Rotation_{Baseline}$ to the $Rotation_{Poststim}$ block for the tTIS [$t(45)=3.99$, $p_{tukey}=.003$] and tACS [$t(45)=3.47$, $p_{tukey}=.014$] groups. In contrast, the sham group did not exhibit a change in ERD [$t(45)=0.251$, $p_{tukey}=1.00$] (see Figure 3). To further titrate if the increases in ERD differed between stimulation groups, the change measure ΔERD was analyzed. This revealed a significant difference among the stimulation groups [$F(2,45)=4.80$, $p=.013$, $\eta_p^2=0.176$], as was expected based on the significant interaction effect in the prior rmANOVA. Post-hoc analysis revealed that both the tTIS [$t(45)=2.91$, $p_{tukey}=.015$] and tACS [$t(45)=2.48$, $p_{tukey}=.043$] groups experienced significantly greater ERD increases compared to the sham group, with no notable difference between tTIS and tACS [$t(45)=0.52$, $p_{tukey}=.861$]. This suggests that both verum stimulations showed increased ERDs compared to sham, while no significant differences between verum stimulation conditions were evident.

Additionally, we performed a correlational analysis to determine if an increase in ERD values corresponded with an improvement in behavioral performance. This did not reveal a significant correlation between ΔAccuracy and ΔERD [$r(46)=.095$, $p=.259$] (see Figure 2e), nor between ΔRT and ΔERD [$r(46)=.021$, $p=.556$] (see Figure 2f). This leads to the conclusion that the change in ERD was not accompanied by a change in behavioral performance.

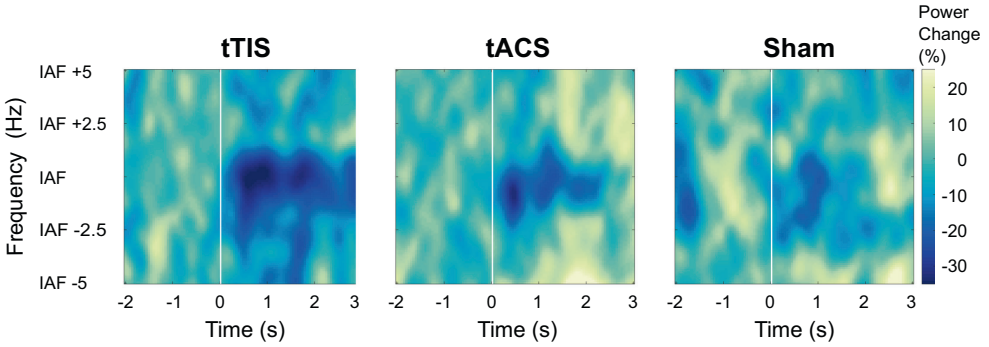

FIGURE 3 Time-Frequency Representations (TFRs) depicting changes in ERD: Contrasted is power at the IAF between the Baseline and the Poststim block. TFRs were aligned at the IAF and averaged across participants within each stimulation group. The reference period for relative baseline correction was defined as the range from −2 to 0 before stimulus onset, as indicated by the white bar. The verum stimulation groups (tTIS, tACS), exhibit a distinct decrease in power around the IAF in the poststim block compared to the baseline block, resulting in increased ERD. The sham group does not exhibit this change in ERD.

3.6 | Exploratory analyses

Analyzing only ERDs has the disadvantage that one key information is missing: As the ERD is itself a ratio between the power in a reference period (i.e., the time period before stimulus presentation) and test period (i.e., the time period after stimulus presentation), it remains unclear if a rise in ERD is driven by a rise in power in the reference period, a loss of power in the test period or a combination of both. To test this, alpha power (IAF + −1 Hz) was extracted for each trial for the reference period (2 to 0 s before stimulus presentation) and test period (0 to 3 s after stimulus presentation) and averaged for each block. Subsequently, we calculated changes in task alpha activity based on the $Rotation_{Baseline}$ and the $Rotation_{Poststim}$ blocks ($\Delta Alpha = Alpha_{Poststim} - Alpha_{Baseline}$) separately for both the reference period and the test period. Then, we performed correlational analyses between ΔERD and ΔAlpha separately for the reference and the test period. This revealed a trend for a positive correlation between ΔAlpha in the reference period with ΔERD [$r(46) = 0.279$, $p = .055$] (see Figure 4b), but no significant correlation between ΔAlpha in the test period with ΔERD [$r(46) = -0.181$, $p = .217$] (see Figure 4a). This suggests that the changes in ERD are more likely due to power changes in the reference period than in the test period.

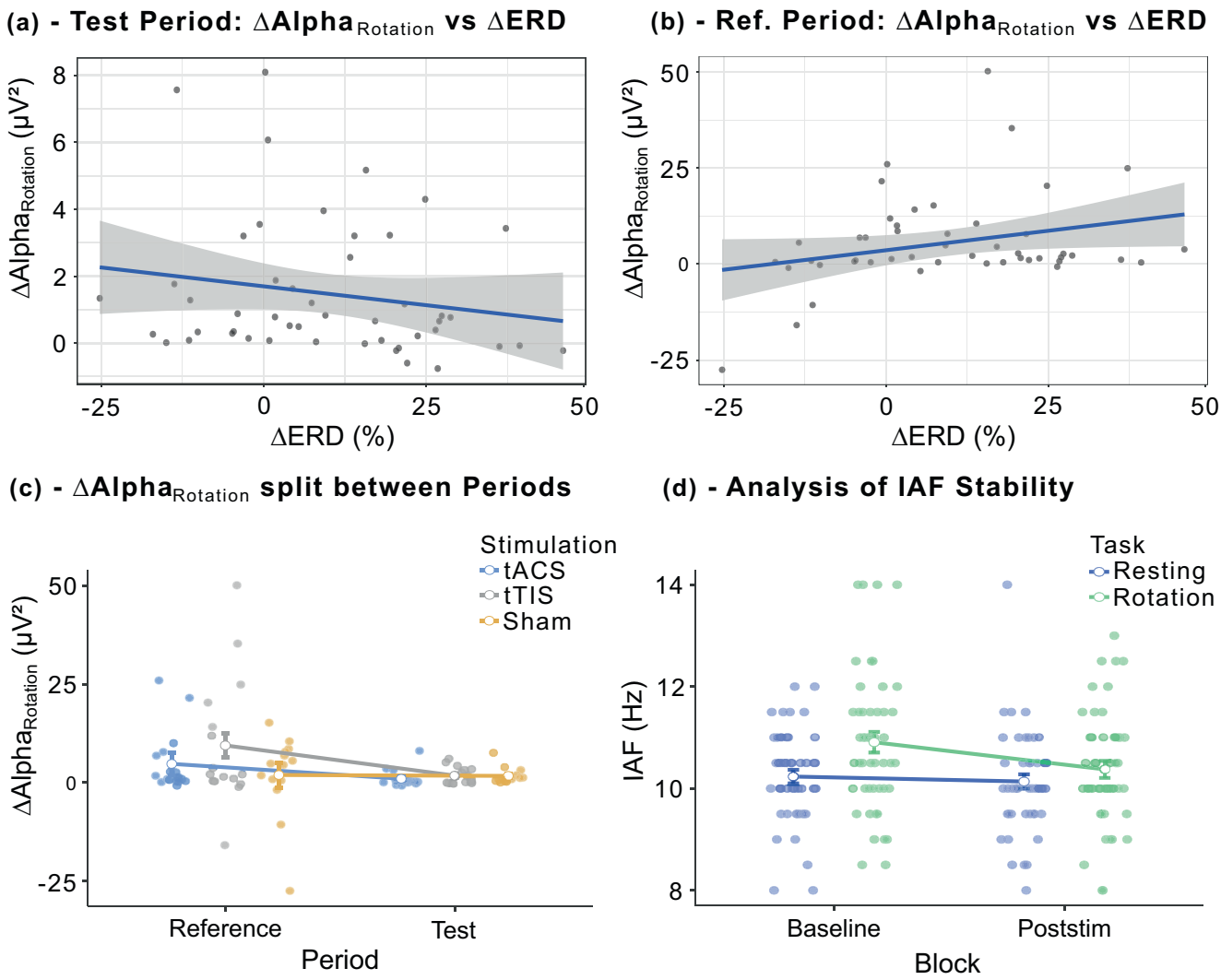

FIGURE 4 Overview of Exploratory Results. (a, b) Correlational analysis of ERD and alpha activity changes. Analysis focused on the difference in ERD (ΔERD) and alpha activity changes ($\Delta Alpha = Alpha_{Poststim} - Alpha_{Baseline}$) during (a) the test period (0 to 3 s after stimulus presentation) and (b) the reference period (−2 to 0 s before stimulus presentation). Results indicated no significant correlation between ΔAlpha in the test period and ΔERD, but a trending significance was observed between ΔAlpha in the reference period and ΔERD. (c) Analysis of alpha changes during mental rotation split between reference and test periods. This indicates that alpha increases were significantly higher in the reference period compared to the test period. There was no difference between stimulation groups. (d) Analysis of IAF across tasks and blocks. The IAF remained stable in resting blocks, whereas in rotation tasks, it was significantly elevated compared to resting blocks and showed a significant slowing from baseline to the poststimulation block.

To further titrate stimulation effects on $\Delta Alpha_{Rotation}$, we fed it into an rmANOVA with the factors *Period* (Reference, Test) and the between-subject factor *Stimulation* (tACS, tTIS, sham). This revealed a significant main effect *Period* [$F(1,45) = 6.10$, $p = .017$, $\eta_p^2 = 0.119$, $\eta_G^2 = 0.052$] which is based on a significantly higher alpha increase in the reference period than in the test period (see Figure 4c). However, neither the main effect *Stimulation* [$F(2,45) = 1.36$, $p = .267$, $\eta_p^2 = 0.057$, $\eta_G^2 = 0.035$] nor the interaction effect *Period* × *Stimulation* [$F(1,45) = 1.76$, $p = .183$, $\eta_p^2 = 0.073$, $\eta_G^2 = 0.031$] reached significance. This suggests that although the reference period appears to be the primary factor influencing ERD changes, attributing the stimulation effect exclusively to either the reference or test period is overly simplistic. It likely indicates a complex interplay between stimulation effects and alpha activity alterations across both periods.

Further, we examined the changes in IAF over the course of the experiment aiming to assess the stability of IAF and to post-hoc verify the accuracy of the stimulation frequency. IAFs were extracted from the baseline blocks ($Rest_{Baseline}$, $Rotation_{Baseline}$) and the poststimulation blocks ($Rest_{Poststim\ 3}$, $Rotation_{Poststim}$). Descriptive analysis revealed varying IAF frequencies [$M_{RestBaseline} = 10.2$ Hz, $SD_{RestBaseline} = 0.91$ Hz, $M_{RestPoststim} = 10.1$ Hz, $SD_{RestPoststim} = 0.99$ Hz, $M_{RotationBaseline} = 10.9$ Hz, $SD_{RotationBaseline} = 1.38$ Hz, $M_{RotationPoststim} = 10.4$ Hz, $SD_{RotationPoststim} = 1.14$ Hz]. To analyze these differences, we employed an rmANOVA with within-subject factors of *Block* (Baseline, Poststim) and *Task* (Resting, Rotation). This revealed a significant main effect of *Block* on IAF [$F(1,47) = 17.71$, $p < .001$, $\eta_p^2 = 0.274$, $\eta_G^2 = 0.019$], attributed to a slowing of IAF from baseline to poststim blocks (see Figure 4d). Additionally, a significant main effect of *Task* was observed [$F(1,47) = 18.08$, $p < .001$, $\eta_p^2 = 0.278$, $\eta_G^2 = 0.044$], indicating a faster IAF during the mental rotation task compared to the resting block. There was no significant interaction of *Block* × *Task* [$F(1,47) = 1.92$, $p = .172$, $\eta_p^2 = 0.039$, $\eta_G^2 = 0.008$].

4 | DISCUSSION

To this day, studies researching in vivo effects of tTIS are still sparse, which is true for murine models but especially in humans. Our current study contributes a proof-of-concept and new evidence for the neuromodulatory effect of tTIS in humans. We demonstrate an outlasting electrophysiological effect of tTIS in the form of an increase of ERD in the alpha range during a mental rotation task. Building on prior work that demonstrated the neuromodulatory potential of tACS on parieto-occipital alpha activity (Kasten et al., 2018; Kasten & Herrmann, 2017), we extend these findings to tTIS. Though contrary to our hypothesis, we observed no stimulation effect on mental rotation performance or resting alpha activity.

To further clarify the specific impact of the stimulation on ERD, we have conducted an additional analysis aimed at determining whether the stimulation effect could be attributed distinctly to alpha changes either in the reference or the test period or a combination of both (as discussed in Kasten & Herrmann, 2017). This analysis uncovered a significantly larger change in alpha power during the reference period than in the test period. Though importantly, we observed no interaction effect between the period-specific changes in alpha activity and the stimulation applied, which stands in contrast to the distinct interaction effect we identified between ERD changes and the stimulation. This suggests that while the alterations in ERD are primarily attributable to the reference period, these alterations alone cannot fully account for the effects of the stimulation. One possible explanation may be that the stimulation effect does not originate from changes in either period individually, but rather from an interaction between changes occurring in both periods.

Even though no specific stimulation effect was evident in the reference period, our correlational analysis suggests that the significant increase in alpha activity during this period might be the primary influence on the overall ERD changes observed. This elevated alpha activity sets the stage for a more marked drop in alpha activity when the stimulus is presented, suggesting that the dynamics within the reference period play a crucial role in shaping the brain's oscillatory response to stimuli. Generally speaking, the ERD is well known to be associated with task performance (Haegens et al., 2011; Kasten & Herrmann, 2017; Klimesch et al., 2003; Neubauer et al., 1995). Klimesch et al. (2003) used repetitive transcranial magnetic stimulation (rTMS) to apply stimulation time-locked to the reference period, in turn boosting alpha activity to result in an increase in ERD and task performance. Though they did not measure ERD, studies by Zoefel et al. (2011) and Hanslmayr et al. (2005) used neurofeedback training to demonstrate a performance advantage of increased parieto-occipital alpha activity on a mental rotation task. The rationale is based on the presumption that alpha range oscillations play a crucial role in regulating information flow. Specifically, high alpha oscillations represent the natural resting or idling state of certain brain areas. However, when a region becomes relevant to a task, alpha oscillations decrease, signaling that the area is transitioning to a state of active information processing, with the change in alpha activity quantified by ERD.

However, in our study, we did not observe an improvement in behavioral performance despite the increased ERD. One potential explanation for this

discrepancy is the presence of a behavioral ceiling effect. This is particularly evident in task accuracy, where all groups achieved near-perfect hit rates in the poststim block. This indicates that participants, including those in the sham group, performed the task with ease, leaving little room for the potential enhancing effect of the stimulation to manifest in improved performance. Thus, the comparable improvement in task performance across all three group rather indicates a significant learning effect, covering any specific effects of stimulation. To detect the specific benefits of stimulation on mental rotation, future studies should consider increasing the task difficulty. Interestingly, our findings, which indicate no significant effect of stimulation on reaction times, are consistent with existing literature that also did not observe a performance improvement following an increase in alpha activity (Klimesch et al., 2003).

A potential limitation in measuring stimulation effects during the mental rotation task is the experimental design, which may have led to an underestimation of the true stimulation effects. Specifically, our experimental design positioned the $Rest_{Poststim\ 2}$ Block in between the $Rotation_{Stimulation}$ and $Rotation_{Poststimulation}$ Blocks, resulting in a 5-minute delay between applied stimulation and measuring its effect on mental rotation. We cannot rule out that the stimulation effect is only strongest immediately after stimulation; thus, this delay could theoretically mean that the peak stimulation impact was not fully present during the $Rotation_{Poststimulation}$ block. Future studies should prioritize examining stimulation effects on mental rotation tasks by immediately following stimulation with a task to accurately assess the outcomes.

Contrary to our initial hypothesis, we did not observe any stimulation effect on alpha activity during the vigilance task. Though the alpha activity experienced an increase between resting blocks, the rate of this increase was consistent across all stimulation groups, indicating that the tTIS and tACS groups did not exhibit greater increases in alpha activity compared to the sham group. This suggests that the observed increase in alpha power over time might be attributed to an increase in mental fatigue throughout the experiment, which is known to increase alpha activity (Hsu & Wang, 2013; Käthner et al., 2014; Trejo et al., 2015), rather than a direct effect of the stimulation. In relation to tACS, our results did not align with the expected increase in alpha activity following parieto-occipital tACS, a well-documented effect in existing literature (Kasten et al., 2016; Kasten & Herrmann, 2017; Neuling et al., 2013; Vossen et al., 2015; Zaehle et al., 2010). Several reasons could account for the absence of observed stimulation effects. One consideration is the electrode montage's efficacy in targeting alpha oscillation generators. Despite using an established tACS montage and verifying field strengths at the region of interest (ROI) with SimNIBS simulations, the possibility of a non-optimal montage cannot be entirely dismissed. Furthermore, uncontrolled intra- and interindividual variations, including suboptimal brain states at the time of stimulation (Bergmann, 2018), could have influenced our results.

However, these potential explanations do not fully account for the discrepancy for a lack of stimulation effect on resting alpha activity and our observed stimulation effect on ERD or task-related alpha activity. It is possible that the alternation between resting and rotation blocks introduced significant interference, given the rotation task's requirement for constant adjustments in alpha activity. This hypothesis is supported by similar findings from Kasten and Herrmann (2017), who employed a similar design featuring alternating rest and task phases and were not able to demonstrate changes in resting alpha activity but were able to observe alterations in task-related alpha activity. Still, our study leaves open questions regarding the precise relationship between stimulation and alpha activity, underlining the necessity for further research to disentangle these complex dynamics.

While computational modeling studies have shown promising outcomes, indicating that tTIS can lead to neuronal entrainment (Karimi et al., 2019; Lee, 2021; Su et al., 2021; Zhu et al., 2022), some research highlights the potential for enhancing its effectiveness. These studies specifically propose enhancing tTIS by adopting an electrode configuration that utilizes multiple pairs of electrodes. This approach aims to increase the intensity at the target site and improve focality, potentially amplifying stimulation efficacy (Cao & Grover, 2019; Howell & McIntyre, 2021; Huang et al., 2020; Huang & Parra, 2019; Zhu et al., 2019). To validate these theoretical improvements, future research should investigate these strategies through in vivo studies.

A potential limitation of this study is the observed lack of robustness of the IAF. The aim of this study was to apply stimulation at each subject's IAF, as the Arnold tongue principle suggests that the efficacy of oscillatory stimulation can be enhanced when the target frequency and the applied frequency are as close as possible (Huang et al., 2021; Kasten et al., 2019; Schutter & Wischnewski, 2016). However, our exploratory analysis revealed that IAF fluctuates over the course of the experiment, a finding that aligns with other studies (Benwell et al., 2019; Stecher et al., 2017; Vossen et al., 2015). In this study, we estimated the IAF based on resting-state activity in the initial baseline resting block. Hence, the optimal stimulation frequency might have shifted by the time stimulation began. Furthermore, these studies suggest

that IAF can vary depending on whether the neuronal system is at rest or under load. Accordingly, we might have misestimated the IAF for the mental rotation task, which led to the application of non-optimal stimulation frequencies. Future studies should consider (1) extracting the IAF immediately before the application of stimulation and (2) using a task that closely resembles the cognitive demands of the target task, to reduce the mismatch between stimulation and target frequency and thus enhance stimulation efficacy. Additionally, adopting closed-loop methods (Karabanov et al., 2016) that adjust stimulation parameters in real-time based on the current brain state (Bergmann, 2018) could offer a more sophisticated approach to optimizing stimulation effectiveness.

In our study, we employed an inactive control stimulation (sham stimulation which was applied for only 30 seconds), which allowed us to differentiate between stimulation effects and effects not based on electrostimulation. Some other tTIS studies (Conta et al., 2022; Wessel et al., 2023) instead opt for an active control condition, where a high-frequency condition without a frequency offset ($\Delta f = f_1 - f_2 = 2000\,\text{Hz} - 2000\,\text{Hz} = 0\,\text{Hz}$) is utilized, allowing to measure stimulation effects specific to the amplitude modulation and potential confounding effects due to the high-frequency signal of the stimulation. Indeed, recent studies are debating a potential effect of the high-frequency signal in the form of a conduction block in off-target areas, which should be considered (Mirzakhalili et al., 2020; Wang et al., 2023), though this is mainly when using high-intensity, suprathreshold tTIS. However, an active control is not necessary to differentiate between entrainment effects and non-specific stimulation effects. We hypothesized that true entrainment would specifically boost power at the frequency of amplitude modulation (IAF), without affecting neighboring frequencies. In contrast, a non-specific stimulation effect would likely cause a broad increase across the frequency spectrum, raising overall power. Our results, as visualized in Figure 3, confirm that the power increase is indeed localized to the IAF and thus frequency-specific, as per the entrainment principle. Nonetheless, future studies should ideally incorporate both an active as well as an inactive control condition to measure and control stimulation-unrelated effects (sham stimulation), non-specific stimulation effects due to the high-frequency signal (active control) as well as true entrainment effects due to the amplitude modulation (verum stimulation).

5 | CONCLUSION

This study offers early electrophysiological evidence for tTIS's in vivo effect on humans. We observed increased ERD during a mental rotation task after both tTIS and tACS stimulation, but no increase in alpha power at rest. This indicates that manifestation of tTIS's effects requires an active task-engaged neuronal network. Although higher ERD has been associated with enhanced information processing in prior studies, we did not see this translate to improved performance in the mental rotation task, likely due to a ceiling effect. Future research should focus on identifying the best stimulation parameters and brain states for modulating behavior through tACS and tTIS.

AUTHOR CONTRIBUTIONS

Carsten Thiele: Conceptualization; data curation; formal analysis; investigation; methodology; software; visualization; writing – original draft. **Katharina S. Rufener:** Conceptualization; writing – review and editing. **Stefan Repplinger:** Software; writing – review and editing. **Tino Zaehle:** Project administration; resources; writing – review and editing. **Philipp Ruhnau:** Conceptualization; funding acquisition; methodology; project administration; resources; supervision; writing – review and editing.

ACKNOWLEDGMENTS

We thank all members of the Zaehle Lab for their valuable contributions to discussions. We would furthermore like to thank two anonymous reviewers for their helpful comments on an earlier version of this document.

FUNDING INFORMATION

This study was supported by the federal state of Saxony-Anhalt and the European Regional Development Fund (ERDF) in the Center for Behavioral Brain Sciences (CBBS, ZS/2016/04/78113). The funding source had no direct involvement in this study.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data sets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

DECLARATION OF AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT-4 in order to improve readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

ORCID

Philipp Ruhnau https://orcid.org/0000-0001-6546-7312

REFERENCES

Abd Hamid, A. I., Gall, C., Speck, O., Antal, A., & Sabel, B. A. (2015). Effects of alternating current stimulation on the healthy and diseased brain. *Frontiers in Neuroscience*, *9*, 391.

Alexander, M. L., Alagapan, S., Lugo, C. E., Mellin, J. M., Lustenberger, C., Rubinow, D. R., & Fröhlich, F. (2019). Double-blind, randomized pilot clinical trial targeting alpha oscillations with transcranial alternating current stimulation (tACS) for the treatment of major depressive disorder (MDD). *Translational Psychiatry*, *9*(1), 1–12.

Antal, A., Ambrus, G. G., & Chaieb, L. (2014). The impact of electrical stimulation techniques on behavior. *Wiley Interdisciplinary Reviews: Cognitive Science*, *5*(6), 649–659.

Beatti, A., Rayner, A., Chipchase, L., & Souvlis, T. (2011). Penetration and spread of interferential current in cutaneous, subcutaneous and muscle tissues. *Physiotherapy*, *97*(4), 319–326. https://doi.org/10.1016/j.physio.2011.01.008

Benwell, C. S. Y., London, R. E., Tagliabue, C. F., Veniero, D., Gross, J., Keitel, C., & Thut, G. (2019). Frequency and power of human alpha oscillations drift systematically with time-on-task. *NeuroImage*, *192*, 101–114.

Berger, A., Pixa, N. H., Steinberg, F., & Doppelmayr, M. (2018). Brain oscillatory and hemodynamic activity in a bimanual coordination task following transcranial alternating current stimulation (tACS): A combined EEG-fNIRS study. *Frontiers in Behavioral Neuroscience*, *12*, 67.

Bergmann, T. O. (2018). Brain state-dependent brain stimulation. *Frontiers in Psychology*, *9*, 2108.

Brown, C. C. (1975). Electroanesthesia and electrosleep. *The American Psychologist*, *30*(3), 402–410.

Brunelin, J., Adam, O., & Mondino, M. (2022). Recent advances in noninvasive brain stimulation for schizophrenia. *Current Opinion in Psychiatry*, *35*(5), 338–344.

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., & Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *International Journal of Neuropsychopharmacology*, *14*(8), 1133–1145.

Brunoni, A. R., Valiengo, L., Baccaro, A., Zanão, T. A., de Oliveira, J. F., Goulart, A., Boggio, P. S., Lotufo, P. A., Benseñor, I. M., & Fregni, F. (2013). The sertraline vs. electrical current therapy for treating depression clinical study: Results from a factorial, randomized, controlled trial. *JAMA Psychiatry*, *70*(4), 383–391.

Cao, J., & Grover, P. (2019). STIMULUS: Noninvasive dynamic patterns of neurostimulation using Spatio-temporal interference. *IEEE Transactions on Biomedical Engineering*, *67*(3), 726–737.

Cao, J., Doiron, B., Goswami, C., & Grover, P. (2020). The mechanics of temporal interference stimulation. *bioRxiv*. https://doi.org/10.1101/2020.04.23.051870

Cassarà, A. M., Newton, T. H., Zhuang, K., Regel, S. J., Achermann, P., Kuster, N., & Neufeld, E. (2022). Safety recommendations for temporal interference stimulation in the brain. *bioRxiv*. https://doi.org/10.1101/2022.12.15.520077

Chen, X., Bin, G., Daly, I., & Gao, X. (2013). Event-related desynchronization (ERD) in the alpha band during a hand mental rotation task. *Neuroscience Letters*, *541*, 238–242.

Cho, H., Razza, L. B., Borrione, L., Bikson, M., Charvet, L., Dennis-Tiwary, T. A., Brunoni, A. R., & Sudbrack-Oliveira, P. (2022). Transcranial electrical stimulation for psychiatric disorders in adults: A primer. *Focus (American Psychiatric Publishing)*, *20*(1), 19–31.

Conta, J. v., Kasten, F. H., Schellhorn, K., Ćurčić-Blake, B., Aleman, A., & Herrmann, C. S. (2022). Benchmarking the effects of transcranial temporal interference stimulation (tTIS) in humans. *Cortex*, *154*, 299–310.

Conta, J. v., Kasten, F. H., Ćurčić-Blake, B., Aleman, A., Thielscher, A., & Herrmann, C. S. (2021). Interindividual variability of electric fields during transcranial temporal interference stimulation (tTIS). *Scientific Reports*, *11*(1), 20357.

Donoghue, T., Haller, M., Peterson, E. J., Varma, P., Sebastian, P., Gao, R., Noto, T., Lara, A. H., Wallis, J. D., Knight, R. T., Shestyuk, A., & Voytek, B. (2020). Parameterizing neural power spectra into periodic and aperiodic components. *Nature Neuroscience*, *23*(12), 1655–1665.

Doppelmayr, M., Klimesch, W., Stadler, W., Pöllhuber, D., & Heine, C. (2002). EEG alpha power and intelligence. *Intelligence*, *30*(3), 289–302.

Esmaeilpour, Z., Kronberg, G., Reato, D., Parra, L. C., & Bikson, M. (2021). Temporal interference stimulation targets deep brain regions by modulating neural oscillations. *Brain Stimulation*, *14*(1), 55–65.

Faul, F., Erdfelder, E., Lang, A.-G., & Buchner, A. (2007). G*power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, *39*(2), 175–191.

Fertonani, A., Ferrari, C., & Miniussi, C. (2015). What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. *Clinical Neurophysiology*, *126*(11), 2181–2188.

Foxe, J. J., & Snyder, A. C. (2011). The role of alpha-band brain oscillations as a sensory suppression mechanism during selective attention. *Frontiers in Psychology*, *2*, 154.

Gabriel, S., Lau, R. W., & Gabriel, C. (1996). The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz. *Physics in Medicine & Biology*, *41*(11), 2251–2269.

Ganis, G., & Kievit, R. (2015). A new set of three-dimensional shapes for investigating mental rotation processes: Validation data and stimulus set. *Journal of Open Psychology Data*, *3*, 1–7. https://doi.org/10.5334/jopd.ai

Goats, G. C. (1990). Interferential current therapy. *British Journal of Sports Medicine*, *24*(2), 87–92. https://doi.org/10.1136/bjsm.24.2.87

Grossman, N., Bono, D., Dedic, N., Kodandaramaiah, S. B., Rudenko, A., Suk, H.-J., Cassara, A. M., Neufeld, E., Kuster, N., & Tsai, L.-H. (2017). Noninvasive deep brain stimulation via temporally interfering electric fields. *Cell*, *169*(6), 1029–1041. e16.

Grossman, N., Okun, M. S., & Boyden, E. S. (2018). Translating temporal interference brain stimulation to treat neurological and psychiatric conditions. *JAMA Neurology*, *75*(11), 1307–1308.

Grover, S., Wen, W., Viswanathan, V., Gill, C. T., & Reinhart, R. M. G. (2022). Long-lasting, dissociable improvements in working memory and long-term memory in older adults with repetitive neuromodulation. *Nature Neuroscience*, *25*(9), 1237–1246.

Guleyupoglu, B., Schestatsky, P., Edwards, D., Fregni, F., & Bikson, M. (2013). Classification of methods in transcranial electrical stimulation (tES) and evolving strategy from historical approaches to contemporary innovations. *Journal of Neuroscience Methods*, *219*(2), 297–311.

Haegens, S., Nácher, V., Luna, R., Romo, R., & Jensen, O. (2011). α-Oscillations in the monkey sensorimotor network influence discrimination performance by rhythmical inhibition of neuronal spiking. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(48), 19377–19382.

Hanslmayr, S., Sauseng, P., Doppelmayr, M., Schabus, M., & Klimesch, W. (2005). Increasing individual upper alpha power by neurofeedback improves cognitive performance in human subjects. *Applied Psychophysiology and Biofeedback*, *30*(1), 1–10.

Herrington, T. M., Cheng, J. J., & Eskandar, E. N. (2016). Mechanisms of deep brain stimulation. *Journal of Neurophysiology*, *115*(1), 19–38.

Herrmann, C. S., & Strüber, D. (2017). What can transcranial alternating current stimulation tell us about brain oscillations? *Current Behavioral Neuroscience Reports*, *4*(2), 128–137.

Herrmann, C. S., Strüber, D., Helfrich, R. F., & Engel, A. K. (2016). EEG oscillations: From correlation to causality. *International Journal of Psychophysiology*, *103*, 12–21.

Howell, B., & McIntyre, C. C. (2021). Feasibility of interferential and pulsed transcranial electrical stimulation for neuromodulation at the human scale. *Neuromodulation*, *24*(5), 843–853.

Hoy, K. E., Bailey, N., Arnold, S., Windsor, K., John, J., Daskalakis, Z. J., & Fitzgerald, P. B. (2015). The effect of γ-tACS on working memory performance in healthy controls. *Brain and Cognition*, *101*, 51–56.

Hsu, B.-W., & Wang, M.-J. J. (2013). Evaluating the effectiveness of using electroencephalogram power indices to measure visual fatigue. *Perceptual and Motor Skills*, *116*(1), 235–252.

Hsu, G., Farahani, F., & Parra, L. C. (2021). Cutaneous sensation of electrical stimulation waveforms. *Brain Stimulation*, *14*(3), 693–702.

Huang, W. A., Stitt, I. M., Negahbani, E., Passey, D. J., Ahn, S., Davey, M., Dannhauer, M., Doan, T. T., Hoover, A. C., Peterchev, A. V., Radtke-Schuller, S., & Fröhlich, F. (2021). Transcranial alternating current stimulation entrains alpha oscillations by preferential phase synchronization of fast-spiking cortical neurons to stimulation waveform. *Nature Communications*, *12*(1), 3151.

Huang, Y., & Parra, L. C. (2019). Can transcranial electric stimulation with multiple electrodes reach deep targets? *Brain Stimulation*, *12*(1), 30–40.

Huang, Y., Datta, A., & Parra, L. C. (2020). Optimization of interferential stimulation of the human brain with electrode arrays. *Journal of Neural Engineering*, *17*(3), 36023.

Hutcheon, B., & Yarom, Y. (2000). Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends in Neurosciences*, *23*(5), 216–222.

Iszak, K., Gronemann, S. M., Meyer, S., Hunold, A., Zschüntzsch, J., Bähr, M., Paulus, W., & Antal, A. (2023). Why temporal inference stimulation may fail in the human brain: A pilot research study. *Biomedicine*, *11*(7), 1813.

JASP Team. (2023). *JASP* (version 0.17) [Computer software].

Jensen, O., & Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: Gating by inhibition. *Frontiers in Human Neuroscience*, *4*, 186.

Karabanov, A., Thielscher, A., & Siebner, H. R. (2016). Transcranial brain stimulation: Closing the loop between brain and stimulation. *Current Opinion in Neurology*, *29*(4), 397–404.

Karimi, F., Attarpour, A., Amirfattahi, R., & Nezhad, A. Z. (2019). Computational analysis of non-invasive deep brain stimulation based on interfering electric fields. *Physics in Medicine and Biology*, *64*(23), 235010.

Kasten, F. H., & Herrmann, C. S. (2017). Transcranial alternating current stimulation (tACS) enhances mental rotation performance during and after stimulation. *Frontiers in Human Neuroscience*, *11*, 2.

Kasten, F. H., Dowsett, J., & Herrmann, C. S. (2016). Sustained aftereffect of α-tACS lasts up to 70 min after stimulation. *Frontiers in Human Neuroscience*, *10*, 245.

Kasten, F. H., Duecker, K., Maack, M. C., Meiser, A., & Herrmann, C. S. (2019). Integrating electric field modeling and neuroimaging to explain inter-individual variability of tACS effects. *Nature Communications*, *10*(1), 5427.

Kasten, F. H., Maess, B., & Herrmann, C. S. (2018). Facilitated event-related power modulations during transcranial alternating current stimulation (tACS) revealed by concurrent tACS-MEG. *eNeuro*, *5*(3), 1–15.

Khadka, N., & Bikson, M. (2023). Noninvasive electrical brain stimulation of the central nervous system. In N. V. Thakor (Ed.), *Handbook of Neuroengineering* (pp. 2101–2133). Springer Nature. https://doi.org/10.1007/978-981-16-5540-1_59

Khan, A., Yuan, K., Bao, S.-C., Ti, C. H. E., Tariq, A., Anjum, N., & Tong, R. K.-Y. (2022). Can transcranial electrical stimulation facilitate post-stroke cognitive rehabilitation? A systematic review and meta-analysis. *Frontiers in Rehabilitation Sciences*, *3*, 795737.

Khatoun, A., Asamoah, B., & Mc, L. M. (2021). A computational modeling study to investigate the use of Epicranial electrodes to deliver interferential stimulation to subcortical regions. *Frontiers in Neuroscience*, *15*, 779271.

Kleiner, M., Brainard, D., & Pelli, D. (2007). *What's new in Psychtoolbox-3?*.

Klimesch, W., Fellinger, R., & Freunberger, R. (2011). Alpha oscillations and early stages of visual encoding. *Frontiers in Psychology*, *2*, 118.

Klimesch, W., Sauseng, P., & Gerloff, C. (2003). Enhancing cognitive performance with repetitive transcranial magnetic stimulation at human individual alpha frequency. *The European Journal of Neuroscience*, *17*(5), 1129–1133.

Klimesch, W., Sauseng, P., & Hanslmayr, S. (2007). EEG alpha oscillations: The inhibition-timing hypothesis. *Brain Research Reviews*, *53*(1), 63–88.

Käthner, I., Wriessnegger, S. C., Müller-Putz, G. R., Kübler, A., & Halder, S. (2014). Effects of mental workload and fatigue on the P300, alpha and theta band power during operation of an ERP (P300) brain-computer interface. *Biological Psychology*, *102*, 118–129.

Lee, S. (2021). *Temporal Interference Stimulation for Focused Stimulation of Deep Structures of Human Brain: A Computer-Based Feasibility Study*: Hanyang University.

Limousin, P., & Foltynie, T. (2019). Long-term outcomes of deep brain stimulation in Parkinson disease. *Nature Reviews Neurology*, *15*(4), 234–242.

Ma, R., Xia, X., Zhang, W., Lu, Z., Wu, Q., Cui, J., Song, H., Fan, C., Chen, X., & Zha, R. (2021). High gamma and Beta temporal

interference stimulation in the human motor cortex improves motor functions. *Frontiers in Neuroscience*, *15*, 800436.

Makeig, S., Debener, S., Onton, J., & Delorme, A. (2004). Mining event-related brain dynamics. *Trends in Cognitive Sciences*, *8*(5), 204–210.

Marchesotti, S., Nicolle, J., Merlet, I., Arnal, L. H., Donoghue, J. P., & Giraud, A.-L. (2020). Selective enhancement of low-gamma activity by tACS improves phonemic processing and reading accuracy in dyslexia. *PLoS Biology*, *18*(9), e3000833.

Miranda, P. C., Mekonnen, A., Salvador, R., & Ruffini, G. (2013). The electric field in the cortex during transcranial current stimulation. *NeuroImage*, *70*, 48–58.

Mirzakhalili, E., Barra, B., Capogrosso, M., & Lempka, S. F. (2020). Biophysics of temporal interference stimulation. *Cell Systems*, *11*(6), 557–572.e5.

Nakayashiki, K., Saeki, M., Takata, Y., Hayashi, Y., & Kondo, T. (2014). Modulation of event-related desynchronization during kinematic and kinetic hand movements. *Journal of Neuroengineering and Rehabilitation*, *11*(1), 90.

Neubauer, A., Freudenthaler, H. H., & Pfurtscheller, G. (1995). Intelligence and spatiotemporal patterns of event-related desynchronization (ERD). *Intelligence*, *20*(3), 249–266.

Neuling, T., Rach, S., & Herrmann, C. S. (2013). Orchestrating neuronal networks: Sustained after-effects of transcranial alternating current stimulation depend upon brain states. *Frontiers in Human Neuroscience*, *7*, 161.

Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, *2011*, 1–9.

Opitz, A., Falchier, A., Yan, C.-G., Yeagle, E. M., Linn, G. S., Megevand, P., Thielscher, A., Deborah, A. R., Milham, M. P., Mehta, A. D., & Schroeder, C. E. (2016). Spatiotemporal structure of intracranial electric fields induced by transcranial electric stimulation in humans and nonhuman primates. *Scientific Reports*, *6*(1), 31236.

Pahor, A., & Jaušovec, N. (2018). The effects of theta and gamma tACS on working memory and electrophysiology. *Frontiers in Human Neuroscience*, *11*, 651.

Paulus, W., Nitsche, M. A., & Antal, A. (2016). Application of transcranial electric stimulation (tDCS, tACS, tRNS): From motor-evoked potentials towards modulation of behaviour. *European Psychologist*, *21*(1), 4–14.

Pfurtscheller, G. (1997). EEG event-related desynchronization (ERD) and synchronization (ERS). *Electroencephalography and Clinical Neurophysiology*, *1*(103), 26.

Pfurtscheller, G., & Aranibar, A. (1977). Event-related cortical desynchronization detected by power measurements of scalp EEG. *Electroencephalography and Clinical Neurophysiology*, *42*(6), 817–826.

Pfurtscheller, G., & Da Silva, F. L. (1999). Event-related EEG/MEG synchronization and desynchronization: Basic principles. *Clinical Neurophysiology*, *110*(11), 1842–1857.

Piao, Y., Ma, R., Weng, Y., Fan, C., Xia, X., Zhang, W., Zeng, G. Q., Wang, Y., Lu, Z., Cui, J., Wang, X., Gao, L., Qiu, B., & Zhang, X. (2022). Safety evaluation of employing temporal interference transcranial alternating current stimulation in human studies. *Brain Sciences*, *12*(9), 1–10.

Pinault, D. (2017). A neurophysiological perspective on a preventive treatment against schizophrenia using transcranial electric stimulation of the corticothalamic pathway. *Brain Sciences*, *7*(4), 34.

Pini, L., Pizzini, F. B., Boscolo-Galazzo, I., Ferrari, C., Galluzzi, S., Cotelli, M., Gobbi, E., Cattaneo, A., Cotelli, M. S., Geroldi, C., Zanetti, O., Corbetta, M., van den Heuvel, M., Frisoni, G. B., Manenti, R., & Pievani, M. (2022). Brain network modulation in Alzheimer's and frontotemporal dementia with transcranial electrical stimulation. *Neurobiology of Aging*, *111*, 24–34.

Rajji, T. K. (2019). Transcranial magnetic and electrical stimulation in Alzheimer's disease and mild cognitive impairment: A review of randomized controlled trials. *Clinical Pharmacology & Therapeutics*, *106*(4), 776–780.

Rampersad, S., Roig-Solvas, B., Yarossi, M., Kulkarni, P. P., Santarnecchi, E., Dorval, A. D., & Brooks, D. H. (2019). Prospects for transcranial temporal interference stimulation in humans: A computational study. *NeuroImage*, *202*, 116124.

Reato, D., Rahman, A., Bikson, M., & Parra, L. C. (2013). Effects of weak transcranial alternating current stimulation on brain activity—A review of known mechanisms from animal studies. *Frontiers in Human Neuroscience*, *7*, 687.

Reinhart, R. M. G., & Nguyen, J. A. (2019). Working memory revived in older adults by synchronizing rhythmic brain circuits. *Nature Neuroscience*, *22*(5), 820–827.

Riečanský, I., & Katina, S. (2010). Induced EEG alpha oscillations are related to mental rotation ability: The evidence for neural efficiency and serial processing. *Neuroscience Letters*, *482*(2), 133–136.

Rihs, T. A., Michel, C. M., & Thut, G. (2009). A bias for posterior alpha-band power suppression versus enhancement during shifting versus maintenance of spatial attention. *NeuroImage*, *44*(1), 190–199.

Romei, V., Gross, J., & Thut, G. (2012). Sounds reset rhythms of visual cortex and corresponding human visual perception. *Current Biology*, *22*(9), 807–813.

Rufener, K. S., & Zaehle, T. (2021). Dysfunctional auditory gamma oscillations in developmental dyslexia: A potential target for a tACS-based intervention. *Progress in Brain Research*, *264*, 211–232.

Rufener, K. S., Zaehle, T., Oechslin, M. S., & Meyer, M. (2016). 40Hz-transcranial alternating current stimulation (tACS) selectively modulates speech perception. *International Journal of Psychophysiology*, *101*, 18–24.

Sachkov, V. I., Liventsev, N. M., Kuzin, M. I., & Zhukovskii, V. D. (1967). Experiences with combined electronarcosis with interference currents in clinical surgery. In *FM Wageneder, electrotherapeutic sleep and electroanaesthesia*. Excerpta Medica.

Salehinejad, M. A., Nejati, V., Mosayebi-Samani, M., Mohammadi, A., Wischnewski, M., Kuo, M.-F., Avenanti, A., Vicario, C. M., & Nitsche, M. A. (2020). Transcranial direct current stimulation in ADHD: A systematic review of efficacy, safety, and protocol-induced electrical field modeling results. *Neuroscience Bulletin*, *36*(10), 1191–1212.

Salehinejad, M. A., Wischnewski, M., Nejati, V., Vicario, C. M., & Nitsche, M. A. (2019). Transcranial direct current stimulation in attention-deficit hyperactivity disorder: A meta-analysis of neuropsychological deficits. *PLoS One*, *14*(4), e0215095.

Sauseng, P., Klimesch, W., Stadler, W., Schabus, M., Doppelmayr, M., Hanslmayr, S., Gruber, W. R., & Birbaumer, N. (2005). A shift of visual spatial attention is selectively associated with human EEG alpha activity. *The European Journal of Neuroscience*, *22*(11), 2917–2926.

Schuhmann, T., Kemmerer, S. K., Duecker, F., de Graaf, T. A., ten Oever, S., de Weerd, P., & Sack, A. T. (2019). Left parietal tACS at alpha frequency induces a shift of visuospatial attention. *PLoS One*, *14*(11), e0217729.

Schutter, D. J., & Wischnewski, M. (2016). A meta-analytic study of exogenous oscillatory electric potentials in neuroenhancement. *Neuropsychologia*, *86*, 110–118.

Shepard, R. N., & Metzler, J. (1971). Mental rotation of three-dimensional objects. *Science*, *171*(3972), 701–703.

Sherman, M. T., Kanai, R., Seth, A. K., & VanRullen, R. (2016). Rhythmic influence of top–down perceptual priors in the phase of prestimulus occipital alpha oscillations. *Journal of Cognitive Neuroscience*, *28*(9), 1318–1330.

Solomons, C. D., & Shanmugasundaram, V. (2019). A review of transcranial electrical stimulation methods in stroke rehabilitation. *Neurology India*, *67*(2), 417–423.

Song, X., Zhao, X., Zhou, Y., Liu, S., He, F., Liu, Y., & Ming, D. (2019). Typical electrode configuration analysis for temporally interfering deep brain stimulation. *2019 9th International IEEE/EMBS Conference on Neural Engineering (NER)*. https://doi.org/10.1109/NER.2019.8717013

Stecher, H. I., Pollok, T. M., Strüber, D., Sobotka, F., & Herrmann, C. S. (2017). Ten minutes of α-tACS and ambient illumination independently modulate EEG α-power. *Frontiers in Human Neuroscience*, *11*, 257.

Stein, D. J., Fernandes Medeiros, L., Caumo, W., & Torres, I. L. S. (2020). Transcranial direct current stimulation in patients with anxiety: Current perspectives. *Neuropsychiatric Disease and Treatment*, *16*, 161–169.

Stoupis, D., & Samaras, T. (2022). Non-invasive stimulation with temporal interference: Optimization of the electric field deep in the brain with the use of a genetic algorithm. *Journal of Neural Engineering*, *19*(5), 056018. https://doi.org/10.1088/1741-2552/ac89b3

Su, X., Guo, J., Zhou, M., Chen, J., Li, L., Chen, Y., Sui, X., Li, H., & Chai, X. (2021). Computational modeling of spatially selective retinal stimulation with temporally interfering electric fields. *IEEE Transactions on Neural Systems and Rehabilitation Engineering*, *29*, 418–428.

Terasawa, Y., Tashiro, H., Ueno, T., & Ohta, J. (2022). Precise temporal control of interferential neural stimulation via phase modulation. *IEEE Transactions on Bio-Medical Engineering*, *69*(1), 220–228.

The jamovi project. (2022). jamovi (Version 2.3) [Computer Software]. Retrieved from https://www.jamovi.org

Thiele, C., Zaehle, T., Haghikia, A., & Ruhnau, P. (2021). Amplitude modulated transcranial alternating current stimulation (AM-TACS) efficacy evaluation via phosphene induction. *Scientific Reports*, *11*(1), 1–10.

Thielscher, A., Antunes, A., & Saturnino, G. B. (2015). Field modeling for transcranial magnetic stimulation: A useful tool to understand the physiological effects of TMS? *37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*.

Thut, G., Schyns, P. G., & Gross, J. (2011). Entrainment of perceptually relevant brain oscillations by non-invasive rhythmic stimulation of the human brain. *Frontiers in Psychology*, *2*, 170.

Trejo, L. J., Kubitz, K., Rosipal, R., Kochavi, R. L., & Montgomery, L. D. (2015). EEG-based estimation and classification of mental fatigue. *Psychology*, *6*(5), 572–589.

Veniero, D., Vossen, A., Gross, J., & Thut, G. (2015). Lasting EEG/MEG aftereffects of rhythmic transcranial brain stimulation: Level of control over oscillatory network activity. *Frontiers in Cellular Neuroscience*, *9*, 477.

Vogeti, S., Boetzel, C., & Herrmann, C. S. (2022). Entrainment and spike-timing dependent plasticity–a review of proposed mechanisms of transcranial alternating current stimulation. *Frontiers in Systems Neuroscience*, *16*, 827353.

Volkmann, J. (2004). Deep brain stimulation for the treatment of Parkinson's disease. *Journal of Clinical Neurophysiology*, *21*(1), 6–17.

Vossen, A., Gross, J., & Thut, G. (2015). Alpha power increase after transcranial alternating current stimulation at alpha frequency (α-tACS) reflects plastic changes rather than entrainment. *Brain Stimulation*, *8*(3), 499–508.

Vosskuhl, J., Strüber, D., & Herrmann, C. S. (2018). Non-invasive brain stimulation: A paradigm shift in understanding brain oscillations. *Frontiers in Human Neuroscience*, *12*, 211.

Wang, B., Aberra, A. S., Grill, W. M., & Peterchev, A. V. (2023). Responses of model cortical neurons to temporal interference stimulation and related transcranial alternating current stimulation modalities. *Journal of Neural Engineering*, *19*(6), 66047.

Wang, H., Wang, K., Xue, Q., Peng, M., Yin, L., Gu, X., Leng, H., Lu, J., Liu, H., Wang, D., Xiao, J., Sun, Z., Li, N., Dong, K., Zhang, Q., Zhan, S., Fan, C., Min, B., Zhou, A., … Wang, Y. (2022). Transcranial alternating current stimulation for treating depression: A randomized controlled trial. *Brain*, *145*(1), 83–91.

Wessel, M. J., Beanato, E., Popa, T., Windel, F., Vassiliadis, P., Menoud, P., Beliaeva, V., Violante, I. R., Abderrahmane, H., Dzialecka, P., Park, C.-H., Maceira-Elvira, P., Morishita, T., Cassara, A. M., Steiner, M., Grossman, N., Neufeld, E., & Hummel, F. C. (2023). Noninvasive theta-burst stimulation of the human striatum enhances striatal activity and motor skill learning. *Nature Neuroscience*, *26*, 1–12.

Wischnewski, M., Engelhardt, M., Salehinejad, M. A., Schutter, D. J. L. G., Kuo, M.-F., & Nitsche, M. A. (2019). NMDA receptor-mediated motor cortex plasticity after 20 Hz transcranial alternating current stimulation. *Cerebral Cortex*, *29*(7), 2924–2931.

Wischnewski, M., Schutter, D. J. L. G., & Nitsche, M. A. (2019). Effects of beta-tACS on corticospinal excitability: A meta-analysis. *Brain Stimulation*, *12*(6), 1381–1389.

Yang, D., Shin, Y.-I., & Hong, K.-S. (2021). Systemic review on transcranial electrical stimulation parameters and EEG/fNIRS features for brain diseases. *Frontiers in Neuroscience*, *15*, 629323.

Zaehle, T., Rach, S., & Herrmann, C. S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS One*, *5*(11), e13766.

Zaghi, S., Acar, M., Hultgren, B., Boggio, P. S., & Fregni, F. (2010). Noninvasive brain stimulation with low-intensity electrical currents: Putative mechanisms of action for direct and alternating current stimulation. *The Neuroscientist*, *16*(3), 285–307.

Zeng, F.-G., Tran, P., Richardson, M., Sun, S., & Xu, Y. (2019). Human sensation of transcranial electric stimulation. *Scientific Reports*, *9*(1), 1–12.

Zhu, X., Li, Y., Zheng, L., Shao, B., Liu, X., Li, C., Huang, Z.-G., Liu, T., & Wang, J. (2019). Multi-point temporal interference stimulation by using each electrode to carry different frequency currents. *IEEE Access*, *7*, 168839–168848.

Zhu, Z., Xiong, Y., Chen, Y., Jiang, Y., Qian, Z., Lu, J., Liu, Y., & Zhuang, J. (2022). Temporal interference (TI) stimulation boosts functional connectivity in human motor cortex: A comparison study with transcranial direct current stimulation (tDCS). *Neural Plasticity*, *2022*, 7605046.

Zoefel, B., Archer-Boyd, A., & Davis, M. H. (2018). Phase entrainment of brain oscillations causally modulates neural responses to intelligible speech. *Current Biology*, *28*(3), 401–408.e5.

Zoefel, B., Huster, R. J., & Herrmann, C. S. (2011). Neurofeedback training of the upper alpha frequency band in EEG improves cognitive performance. *NeuroImage*, *54*(2), 1427–1431.

Zumer, J. M., Scheeringa, R., Schoffelen, J.-M., Norris, D. G., & Jensen, O. (2014). Occipital alpha activity during stimulus processing gates the information flow to object-selective cortex. *PLoS Biology*, *12*(10), e1001965.

How to cite this article: Thiele, C., Rufener, K. S., Repplinger, S., Zaehle, T., & Ruhnau, P. (2024). Transcranial temporal interference stimulation (tTIS) influences event-related alpha activity during mental rotation. *Psychophysiology*, *00*, e14651. https://doi.org/10.1111/psyp.14651

Journal of Cognitive Enhancement
https://doi.org/10.1007/s41465-024-00304-2

ORIGINAL RESEARCH

Perceptibility and Pain Thresholds in Low- and High-Frequency Alternating Current Stimulation: Implications for tACS and tTIS

Carsten Thiele[1,2] · Cornelius Tamm[1] · Philipp Ruhnau[2,3] · Tino Zaehle[1,2]

Received: 20 October 2023 / Accepted: 15 July 2024

Abstract

Transcranial electrical stimulation (tES) has emerged as a promising tool for neuromodulation, but its application is often limited by the discomfort associated with higher stimulation intensities. Newer variants like transcranial temporal interference stimulation (tTIS) utilize high-frequency alternating currents (≥ 500 Hz) to penetrate deeper brain regions while mitigating perceptual discomfort. This study sought to examine sensation and pain thresholds across various stimulation frequencies of alternating currents, aiming to explore the boundaries of comfortable intensities. Additionally, we sought to evaluate the efficacy of an anesthetizing topical cream in increasing participant comfort and potentially extending the range of tolerable stimulation levels. We recruited 37 participants and applied alternating current stimulation to the head at various frequencies (10 Hz, 20 Hz, 500 Hz, 1000 Hz, and 2000 Hz) to determine intensity-dependent perception and pain thresholds. Additionally, thresholds were determined under the influence of a topical anesthetic. Our findings confirm that as stimulation frequency increases, perceptibility decreases, with higher frequencies allowing a manyfold increase in stimulation intensity before becoming perceptible or causing pain. Additionally, the anesthetizing cream was efficacious in further reducing perceptibility and pain sensations across all frequencies. This study lays the groundwork for future research by establishing comfortable limits for stimulation intensities, particularly in the context of high-frequency stimulation. The reduced perceptibility of high-frequency stimulation, coupled with the effectiveness of anesthetizing creams, enables the administration of higher stimulation intensities for more potent neuromodulatory interventions without causing discomfort.

Keywords Transcranial alternating current stimulation (tACS) · Transcranial electrical stimulation (tES) · Transcranial temporal interference stimulation (tTIS) · Somatosensory perception · Nociception · Topical skin anesthetization

Introduction

Transcranial electrical stimulation (tES) holds significant promise for treating various psychological and neurological conditions. Research has explored its potential applications in depression (Arul-Anandam & Loo, 2009; Vanderhasselt et al., 2015), stroke (Convento et al., 2016; Khan et al., 2022; Solomons & Shanmugasundaram, 2019), and fatigue (Linnhoff et al., 2019; Shirvani et al., 2021) among others (Cho et al., 2022). Beyond therapeutic applications, there is growing interest in leveraging this technology for neurofacilitation to, for example, enhance motoric performance (Chang, 2022; Friehs et al., 2022; Perrey, 2023), working memory (Röhner et al., 2018; Zaehle et al., 2011), and perception (He et al., 2022; Wang et al., 2020) in healthy individuals. However, the efficacy and reliability of these interventions in human participants is often constrained by the intensity of stimulation that can be comfortably administered. As

Philipp Ruhnau and Tino Zaehle shared senior authorship.

✉ Carsten Thiele
carsten.thiele@med.ovgu.de

✉ Philipp Ruhnau
pruhnau@uclan.ac.uk

[1] Department of Neurology, Otto-Von-Guericke-University, University Clinic of Magdeburg, 39120 Magdeburg, Germany

[2] Center for Behavioral Brain Sciences (CBBS), Otto-Von-Guericke-Universität Magdeburg, 39120 Magdeburg, Germany

[3] School of Psychology and Humanities, University of Central Lancashire, Preston PR1 2HE, UK

Published online: 06 August 2024

the intensity increases, participants report sensations that evolve from a mere tingling to pronounced discomfort such as prickling sensations and with sufficient intensity even burning or pain sensations (Fertonani et al., 2015; Hsu et al., 2021; Khadka et al., 2020; Kuhn et al., 2010; McFadden et al., 2011; Palmer et al., 1999; Paneri et al., 2016; Zeng et al., 2019).

Consequently, tES studies in humans usually stay at or below a stimulation intensity of 2 mA (Antal & Paulus, 2013; Bikson et al., 2009), with only very few studies employing higher intensities of up to 4 mA (e.g. Chhatbar et al., 2017; Hsu et al., 2023), albeit this being still a safe stimulation intensity (Antal et al., 2017; Bikson et al., 2016; Chhatbar et al., 2017; Matsumoto & Ugawa, 2017; Nitsche & Bikson, 2017). This may be due to participant compliance issues caused by uncomfortable cutaneous sensations. Sensitivity to stimulation varies based on the method of electrostimulation and specific parameters of stimulation (Ambrus et al., 2010; Fertonani et al., 2015) such as electrode size (Kuhn et al., 2010; Turi et al., 2014) or waveform of stimulation (Baker et al., 1988; Hsu et al., 2021). There is an ongoing debate about the role that current density plays in perceivability. On one hand, larger electrodes result in a lower current density, which means less current impacts each somatosensory receptor, potentially reducing perceivability (Alon et al., 1994; Verhoeven & van Dijk, 2006). On the other hand, larger electrodes cover a greater area, leading to a spatial summation effect—namely, the recruitment of more somatosensory receptors to fire, thereby enhancing perceivability (Higashiyama & Tashiro, 1990; Nielsen & Arendt-Nielsen, 1997). Further, the stimulation duration influences perceptibility due to adaptation processes attenuating sensations. This was, for example, leveraged by Khadka et al. (2020) by gradually increasing intensity over the course of the stimulation in an adaptive procedure, enhancing participant comfort at higher stimulation intensities. When using alternating currents, another key parameter in perceptibility is frequency. Frequencies below 100 Hz, commonly used in transcranial alternating current stimulation (tACS), are more perceptible than higher frequencies (Hsu et al., 2021; Turi et al., 2013; Ward & Robertson, 1998; Zeng et al., 2019) due to the spectral specificity of neurons limiting responsiveness to high frequencies (Anderson & Munson, 1951; Hawkes & Warm, 1960; Hutcheon & Yarom, 2000; Palmer et al., 1999).

A recent advancement in the area of tES research is transcranial temporal interference stimulation (tTIS). Its efficacy in modulating brain activity has been demonstrated in animal studies, highlighting it as a promising new electrostimulation method (Acerbo et al., 2022; Carmona-Barrón et al., 2023; Grossman et al., 2017; Liu et al., 2023; Missey et al., 2021; Song et al., 2021a, 2021b; Sunshine et al., 2021; Zhang et al., 2022). TTIS stands out among other tES methods due to its enhanced stimulation depth. This is achieved through the utilization of two high-frequency alternating currents ($\geq$ 500 Hz), creating an amplitude-modulated signal at the intersection of the two fields (Grossman et al., 2017; Karimi et al., 2019; Mirzakhalili et al., 2020; Song, 2019). This signal is believed to lead to modulation of neuronal activity via entrainment effects. By carefully configuring electrode placements and adjusting the current ratios of the fields, the point of interference—and consequently, the stimulation focus—can be directed deeper into brain regions. This offers the potential to non-invasively achieve neuronal modulation in deep brain regions. However, evidence supporting tTIS's efficacy in humans remains limited, with some studies even casting doubt on its feasibility (Budde et al., 2023; von Conta et al., 2022; Iszak et al., 2023). A network modeling study by Negahbani et al. (2018) as well as single neuron modeling studies (Mirzakhalili et al., 2020; Wang et al., 2023) and an in vitro study by Esmaeilpour et al. (2021) indicate that higher tTIS intensities compared with tACS are needed to achieve similar neuronal modulation. However, other studies argue that the efficacy of tTIS is largely based on network mechanisms (Cao, 2018; Martinez et al., 2023) as well as a gradual depolarization of neurons over time (Cao et al., 2020). In this context, tTIS offers a significant advantage due to its high-frequency stimulation, which makes the stimulation intensities less perceptible, allowing the application of higher intensities without discomfort. This leads to a growing interest in increasing the stimulation intensity to fully leverage the potential of tTIS, positioning it as a promising method for both treatment and research in non-invasive deep brain stimulation (Grossman et al., 2018).

Our study aimed to establish tolerable stimulation intensity ranges for both low-frequency (10, 20 Hz) and high-frequency (500, 1000, 2000 Hz) alternating currents. We selected low frequencies due to their common use in tACS studies and high frequencies for their relevance in tTIS research. By measuring pain thresholds at these frequencies, we sought to provide a reference for determining safe and tolerable maximum stimulation intensities for future tTIS and tACS research. Similarly, with perception thresholds, our goal was to identify sub-perception intensity levels crucial for ensuring effective blinding.

Studies have advocated for the use of anesthetizing skin creams in tES studies (Antal et al., 2017; Guleyupoglu et al., 2014; Liu et al., 2018; McFadden et al., 2011), offering multiple advantages. Foremost, these creams allow for the application of higher stimulation intensities than typically feasible (McFadden et al., 2011). The anesthetized skin diminishes pain perceptions, enhancing participant comfort even at higher stimulation levels. Another significant benefit is the improved blinding of participants. Many studies compare verum (true) stimulation with sham (false/placebo/control) stimulation, where sham stimulation involves applying electrical currents that mimic the cutaneous sensations

(such as tingling or itching) of verum stimulation but differ in key aspects. These differences can include being turned off after a brief period, utilizing a different frequency, or targeting a different region. The purpose of sham stimulation, as opposed to not applying any stimulation at all, is to create a condition that feels similar to the verum condition for participants, making it harder for them to differentiate between the two types of stimulation and maintain participant blinding. Using anesthetization as a complementary approach allows to reduce perceptibility of the verum stimulation, thus facilitating participant blinding (Sheffield et al., 2022). A third advantage is the possibility to control for somatosensory perception as a confounding factor in interpretation of stimulation results. Studies have demonstrated that changes in brain activity can be achieved by somatosensory entrainment due to cutaneous sensations of stimulation (Asamoah et al., 2019; Spooner et al., 2022). Thus, recent studies have begun to control for cutaneous sensations to eliminate somatosensory entrainment as a possible confounding factor (Koganemaru et al., 2020; Turi et al., 2020).

Consequently, the second aim of our study was to investigate the influence of a topical anesthetic skin cream by quantifying its impact on somatosensory perception. This was measured by observing changes in the perception and pain thresholds resulting from the application of the anesthetization. Specifically, we aimed to evaluate the anesthetization's efficacy across various stimulation frequencies, to further support it as a future tool in studies to reduce participants' awareness of stimulation conditions and increase the limits of comfortable stimulation intensities.

Methods

Participants

We recruited 37 participants (12 male, 25 female, mean age = 23.6 years, SD = 3.93 years, range = 18–36 years) for this study. Participants with a history of epileptic seizures, psychiatric or neurological disorders, metal or electric implants in the head, or those on medication affecting the central nervous system were excluded. Prior to the experiment, all participants were briefed about the procedure, potential risks of electrostimulation, and provided written informed consent. The study received approval from the University Clinic of Magdeburg's local ethics committee and adhered to the Declaration of Helsinki guidelines.

Experimental Design

We developed the experimental paradigm using MATLAB (Version 2020a, The MathWorks Inc., Natick, MA, USA) and the Psychtoolbox 3 (Kleiner et al., 2007).

Prior to the experiment, topical skin anesthetization cream with 25 mg/g lidocaine and 25 mg/g prilocaine (Anesderm, Pierre Fabre Dermo-Kosmetic GmbH) was applied to one side of each participant's head, while the opposite side remained untreated, serving as a non-anesthetized control. The side of anesthetization was counterbalanced among participants. The anesthetizing cream remained in place for 15 min to ensure its full effect. Afterwards, it was removed with a dry tissue to be replaced by an electrically conductive gel for the following stimulation.

The experiment comprised two task blocks: the somatosensory perception threshold block and the pain threshold block, with their order counterbalanced among participants to mitigate effects of task order. In the somatosensory perception block, participants were tasked to indicate if they experienced any cutaneous sensations such as tingling or itching during stimulation. During the pain threshold block, participants had to indicate if the stimulation had induced pain in the form of stinging or burning sensations. We emphasize that the staircase procedure only gradually increased stimulation intensity over trials and decreased if pain was reported. This ensured that participants only ever experienced mild pain sensations.

A block was comprised of 10 conditions: Anesthetization (yes/no) by Frequency (10 Hz, 20 Hz, 500 Hz, 1000 Hz, 2000 Hz). While the frequency variable was operationalized through the frequency of the applied alternating current, anesthetization was operationalized based on the location of stimulation—whether stimulation was administered to the anesthetized side of a participant's head or the untreated side. Each condition had its own staircase, resulting in ten individual staircases during a block. We adopted a random interleaved staircase design, wherein each consecutive stimulation was based on a randomly selected condition (see Fig. 1A). This design was implemented specifically to minimize habituation effects, which can occur when sensitivity to stimulation decreases due to the same condition being presented consecutively. By ensuring a varied sequence of frequencies and intensities, we aimed to maintain participant sensitivity and mitigate diminishing responses to the stimulation. The total number of trials needed for a staircase to conclude, depended on how many trials were needed to determine a threshold, i.e., to fulfill one of the conclusion criteria (see "Staircase Procedure" section). After a staircase was concluded, its condition was not presented again. A block ended, when all 10 staircases were concluded.

The starting amplitudes for each condition's staircase varied based on the frequency, aligning with the premise that lower frequencies are generally more perceivable, necessitating it to begin at low intensities. Conversely, higher frequencies are less perceivable, warranting a start at higher intensities to avoid the need for presentation of many unperceivable trials before reaching an intensity level relevant for those

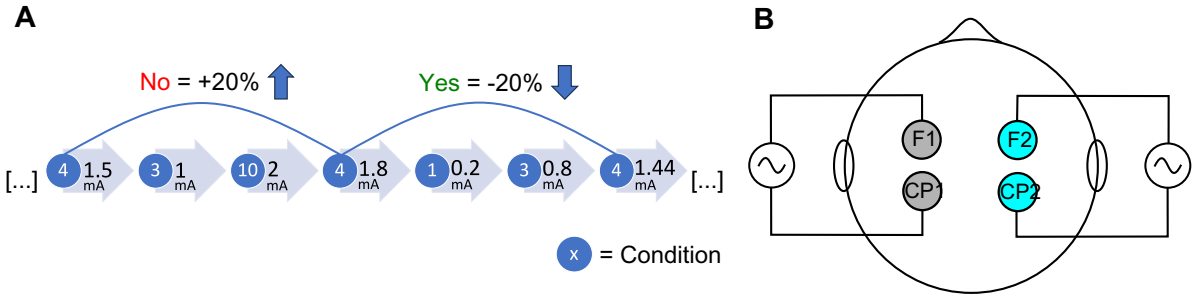

Fig. 1 **A** Consecutive trials exemplified. This illustrates how the stimulation intensity for condition 4 (1000 Hz, not anesthetized) changed depending on the subject's answer. After each trial, a question was posed to the subject (Perception block: "Did you feel the stimulation?"; Pain block: "Did you experience pain during the stimulation?"). Indicating a "No" via button press led to an increase in intensity for future presentations of that condition, whereas a "Yes" decreased the intensity. **B** Electrode montage. In this example, blue electrodes represent the side where anesthetization was applied, while grey electrodes indicate the untreated side. The side on which anesthetization was administered varied, being counterbalanced across participants

frequencies. The starting amplitudes for the conditions were as follows: 10 and 20 Hz: 0.2 mA, 500 Hz: 1 mA, 1000 Hz: 1.5 mA, 2000 Hz: 2 mA.

A trial was comprised of a 3-s countdown which was displayed on a screen in front of the participant, followed by 7 s of stimulation and ended with a self-timed period where participants had to indicate via button press if they felt the stimulation (perception threshold block) or felt pain during stimulation (pain threshold block).

Upon concluding the experiment, participants completed a questionnaire regarding potential side effects, such as lasting pain or headaches (Brunoni et al. 2011). They were then debriefed about the study's objectives and compensated with either course credit or monetarily.

Staircase Procedure

We employed an adaptive 1-up-1-down staircase procedure to determine thresholds (Cornsweet, 1962; Leek, 2001). This method estimates the stimulation strength at which participants would perceive the stimulation (perception threshold) or experience pain (pain threshold) in 50% of trials, by dynamically adjusting stimulation intensities. For example, at the end of a trial, if participants answered with "yes" to the post-stimulation question ("did you feel the stimulation" or "did you experience pain during the stimulation"), the intensity for the future presentation of that condition was decreased by 20%, based on the last given intensity. Conversely, a "no" lead to a 20% increase of intensity. A significant benefit of adaptive staircase procedures is their ability to ensure a high sampling density at and around the most relevant stimulation intensity. This approach prioritizes sampling near the intensity levels where a reversal of answers occurs, while avoiding unnecessary presentation of intensities which are far from the relevant range.

A condition's staircase could conclude in either of the following ways:

(1) After a total of five reversals of "yes/no" responses in a condition. Reversals did not have to be consecutive. The threshold was determined by averaging the last three alternating values in that staircase. To ensure a sufficient number of trials and data collection for accurately pinpointing the thresholds, we chose to require five reversals. We opted to average only the last three reversals because the initial reversals are usually further from the true threshold. In contrast, later reversals tend to be closer, making them more indicative of the actual thresholds (Leek, 2001).
(2) If a condition was presented with a stimulation intensity of 4 mA and received a "no" answer for the third time. Note that "no" answers did not have to be consecutively but were counted over the whole block. This suggests that the actual threshold for inducing sensation or pain in that condition lies above our upper limit of 4 mA. For the purposes of data analysis, we treated these instances as having a threshold of 4 mA, acknowledging that this represents the maximum intensity tested and not the actual somatosensory perception or pain threshold.
(3) If a condition has been presented for the 20th time during that block. For data analysis, the threshold was assumed as the last three alternating values.

Electrical Stimulation

Stimulation was delivered using two independent battery-driven neuroConn Stimulator systems (Advanced DC-Stimulator Plus for temporal interference stimulation, neuroConn GmbH, Ilmenau, Germany) which were connected via a digital to analog converter (Ni USB-6212, National

Instruments, Austin, TX, USA) to a PC and controlled by it using the remote mode of the stimulators. A custom MATLAB script (Version 2020a, The MathWorks Inc., Natick, MA, USA) was used to generate and send the stimulation signal. For stimulation, Ag–AgCl electrodes with a 12-mm diameter (Brain Products, Gilching, Germany) were used, which were affixed to an EEG cap (Easycap, Brain Products, Gilching, Germany). Using the international 10–10 system, electrodes were placed at positions F1 and CP1 for the left stimulation site and at F2 and CP2 for the right stimulation site (see Fig. 1 B). To increase electrode to skin conductivity, we applied a conductive paste (Abralyt 2000 abrasive electrolyte-gel, Brain Products, Gilching, Germany), ensuring impedances remained below 5 kΩ.

Stimulation frequencies were 10 Hz, 20 Hz, 500 Hz, 1000 Hz, and 2000 Hz with a maximum possible intensity of 4 mA. Our rationale for these was as follows: the 2000 Hz and 1000 Hz frequencies were used by Grossman et al. (2017) in their tTIS study and thus reflect proven and efficacious stimulation frequencies. The 500 Hz stimulation frequency is considered to be the lowest frequency feasible for tTIS (Grossman et al., 2017). Additionally, we selected 10 and 20 Hz, standard tACS stimulation frequencies prevalent in many tACS studies, to serve as a reference point to benchmark perception differences between low- and high-frequency stimulation.

Data Analysis

We performed our statistical analysis using Jamovi version 2.3 (The Jamovi Project 2024). To analyze differences between thresholds, we conducted repeated-measures analyses of variance (rmANOVAs) separately for perception- and pain thresholds using within-subject factors Anesthetization (Yes, No) and Frequency (10 Hz, 20 Hz, 500 Hz, 1000 Hz, and 2000 Hz). To ensure that factors in our rmANOVAs conformed to the sphericity assumption, we conducted a Mauchly's test.

For rmANOVA results, we report partial eta squared (η^2_p) to focus on effect size within our chosen design, as well as generalized eta squared (η^2_G) to facilitate comparing effect sizes across studies.

Results

The descriptive outcomes for perception and pain thresholds are illustrated in Fig. 2 and detailed in Table 1. In addition, Table 1 also presents the intensity values converted to current density, based on the 12 mm electrodes employed in our study.

Statistical analysis revealed violations of the sphericity assumption for perception thresholds [*Frequency*: $\chi 2\ (9) = 166.67,\ p < 0.001,\ \varepsilon = 0.49$; *Frequency * Anesthetization*: $\chi 2(9) = 132.29, p < 0.001, \varepsilon = 0.59$] as well as for pain thresholds [*Frequency*: $\chi 2(9) = 105.19, p < 0.001, \varepsilon = 0.47$; *Frequency * Anesthetization*: $\chi 2(9) = 47.96, p < 0.001, \varepsilon = 0.66$]. Given these violations, we adjusted the degrees of freedom using the Greenhouse–Geisser correction to make the test more conservative and control for type I errors.

Results of the rmANOVAs revealed a significant main effect of *Frequency* on perception- [$F(1.95,70.09) = 479.16$, $p < 0.001$, $\eta 2_p = 0.930$, $\eta 2_G = 0.796$] and pain thresholds [$F(1.86,67.11) = 588.70$, $p < 0.001$, $\eta 2_p = 0.942$, $\eta 2_G = 0.844$]. This indicates that higher frequencies lead to increased thresholds for perception and pain, implying that higher stimulation frequencies induce less cutaneous sensations than lower frequencies. This was confirmed using post-hoc analyses: as frequencies increased, so did the thresholds for both perception and pain. This was demonstrated by significant increases across all frequency comparisons for perception [all comparisons $t(36) > 3.22$, $p_{tukey} < 0.001$] and pain thresholds [all comparisons $t(36) > 5.32$, $p_{tukey} < 0.001$], with the sole exception being the pain thresholds between 10 and 20 Hz frequencies, which did not differ significantly [$t(36) = 2.26$, $p_{tukey} = 0.181$]. Additionally, a significant main effect of *Anesthetization* on thresholds was revealed, again for both perception [$F(1,36) = 19.90$, $p < 0.001$, $\eta 2_p = 0.356$, $\eta 2_G = 0.086$] and pain thresholds [$F(1,36) = 26.16$, $p < 0.001$, $\eta 2_p = 0.421$, $\eta 2_G = 0.077$], indicating that the anesthetization reduced cutaneous sensations to the stimulation in both measures. Additionally, an interaction *Frequency * Anesthetization* was observed for both perception [$F(2.36,84.85) = 6.08$, $p = 0.002$, $\eta 2_p = 0.144$, $\eta 2_G = 0.031$] and pain thresholds [$F(2.65,95.31) = 5.96$, $p = 0.001$, $\eta 2_p = 0.142$, $\eta 2_G = 0.023$]. This was due to the efficacy of anesthetization varying based on the stimulation frequency, with higher stimulation frequencies (500 and 1000 Hz) benefitting more from anesthetization than lower frequencies (10 and 20 Hz). At 2000 Hz, however, our analysis found no difference in thresholds between anesthetized and non-anesthetized conditions [perception: $t(36) = 1.47$, $p_{tukey} = 0.894$; pain: $t(36) = 1.09$, $p_{tukey} = 0.983$]. It is important to note that this absence of an anesthetization effect at 2000 Hz is a result of many subjects reaching our study's maximum stimulation of 4 mA in both anesthetized and non-anesthetized conditions, reflecting a limitation of our study setup.

Exploratory, to mitigate potential confounding effects of task order, we repeated the previous rmANOVAs including *Task Order* (pain task first, perception task first) as a between-subject factor. The results indicated that *Task Order* did not significantly affect perception thresholds [$F(1,35) = 0.225$, $p = 0.638$,$\eta 2_p = 0.006$, $\eta 2_G = 0.002$] or pain thresholds [$F(1,35) = 0.015$, $p = 0.902$, $\eta 2_p = 0.010$, $\eta 2_G = 0.004$]. Furthermore, to analyze a potential difference

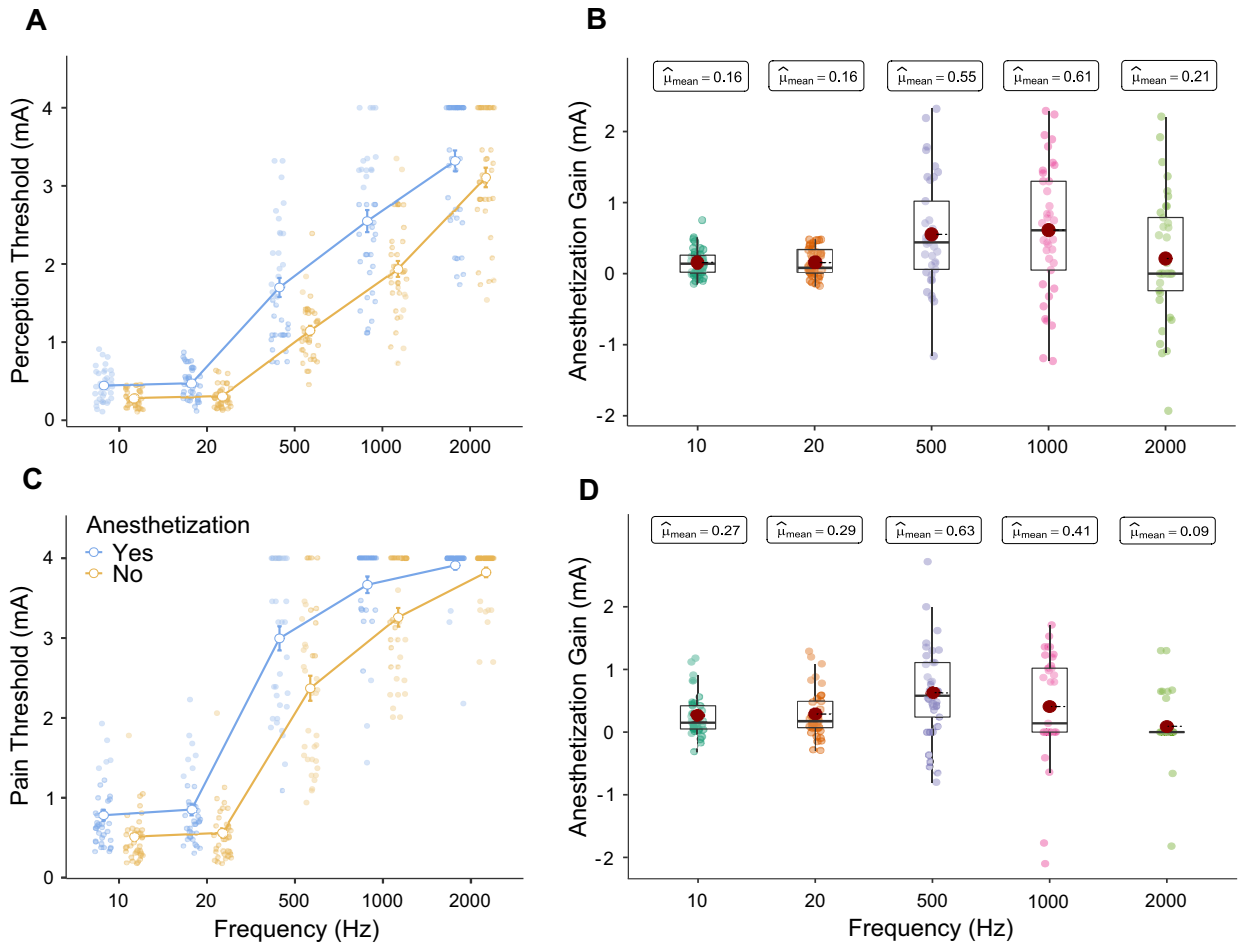

Fig. 2 Stimulation intensity thresholds. **A** Perception thresholds increase with stimulation frequency, indicating that high-frequency stimulation induces less perception. Conditions with topical anesthetization display higher thresholds. **B** Anesthetization allows for higher stimulation intensities before reaching perception thresholds across all frequencies, as indicated by *Anesthetization Gains* ($=\text{Threshold}_{\text{WithAnesthetization}} - \text{Threshold}_{\text{WithoutAnesthetization}}$). Notably, the reduced anesthetization gain at 2000 Hz is due to some participants not perceiving any stimulation at high frequencies, both with and without anesthetization. For analysis, their threshold was standardized to 4 mA, aligning anesthetized and non-anesthetized conditions and reducing observed anesthetization gains. This reflects study constraints more than a decrease in anesthetization effectiveness at higher frequencies. **(C)** Pain thresholds: Higher stimulation frequencies correlate with lower pain thresholds, reflecting the pattern seen in perception thresholds. **(D)** Anesthetization gains for pain thresholds: These gains follow a similar trend to perception thresholds, with the same high-frequency constraints previously noted

between sexes, we repeated the rmANOVAs again with *Sex* (male, female) as a between-subject factor. This analysis revealed a significant effect of *Sex* on pain thresholds [$F(1,35)=4.34$, $p=0.045$, $\eta 2_p=0.110$, $\eta 2_G=0.048$]. Additionally, a significant interaction effect *Frequency* * *Sex* was observed [$F(1.99,69.50)=5.346$, $p=0.007$, $\eta 2_p=0.133$, $\eta 2_G=0.046$], which is descriptively explained by male participants being able to tolerate higher intensities at high frequencies, though this did not reach significance in post-hoc analysis [male vs. female; 10 Hz: *Mean Diff* = 0.01 mA, $t(35)=0.085$, $p_{tukey}=1.000$; 20 Hz: *Mean Diff* = 0.08 mA, $t(35)=0.665$, $p_{tukey}=1.000$; 500 Hz: *Mean Diff* = 0.71 mA, $t(35)=0.665$, $p_{tukey}=0.250$; 1000 Hz: *Mean Diff* = 0.42 mA, $t(35)=2.413$, $p_{tukey}=0.349$; 2000 Hz: *Mean Diff* = 0.12 mA, $t(35)=1.322$, $p_{tukey}=0.942$]. No other effects reached significance. Further, no significant effect of *Sex* on perception thresholds [$F(1,35)=0.097$, $p=0.758$, $\eta 2_p=0.003$., $\eta 2_G=0.001$] could be observed.

Discussion

Current human applications of tES are limited by the maximum intensity that participants can comfortably tolerate. Even though higher intensities are considered safe (Antal et al., 2017; Bikson et al., 2016; Chhatbar et al., 2017; Matsumoto & Ugawa, 2017), the discomfort from skin sensations or pain often restricts their use. The recent introduction of tTIS (Grossman et al., 2017) leverages the fact that high-frequency alternating currents are

Table 1 Descriptive statistics of perception and pain thresholds

	Without anesthetization					With anesthetization				
	10 Hz	20 Hz	500 Hz	1000 Hz	2000 Hz	10 Hz	20 Hz	500 Hz	1000 Hz	2000 Hz
Perception thresholds										
Mean (mA)	0.29	0.31	1.15	1.94	3.11	0.44	0.47	1.70	2.55	3.32
Mean CD (mA/cm^2)	0.26	0.27	1.02	1.72	2.75	0.39	0.42	1.50	2.25	2.94
Median (mA)	0.27	0.28	1.13	1.90	3.07	0.43	0.46	1.58	2.53	4.00
SD (mA)	0.11	0.14	0.38	0.62	0.76	0.21	0.20	0.74	0.86	0.81
Min (mA)	0.11	0.13	0.46	0.73	1.54	0.11	0.12	0.74	1.12	1.74
Max (mA)	0.46	0.64	2.39	3.35	4.00	0.91	0.87	3.32	4.00	4.00
N_{Lim}/N_{Total}	0/37	0/37	0/37	0/37	11/37	0/37	0/37	0/37	4/37	19/37
Pain thresholds										
Mean (mA)	0.51	0.56	2.37	3.26	3.82	0.78	0.85	3.00	3.67	3.91
Mean CD (mA/cm^2)	0.45	0.50	2.10	2.88	3.38	0.69	0.75	2.65	3.25	3.46
Median (mA)	0.44	0.52	2.35	3.21	4.00	0.67	0.71	3.20	4.00	4.00
SD (mA)	0.31	0.35	0.96	0.71	0.37	0.40	0.44	0.92	0.63	0.34
Min (mA)	0.18	0.18	0.94	2.01	2.70	0.33	0.31	1.09	1.44	2.18
Max (mA)	1.78	2.06	4.00	4.00	4.00	1.93	2.23	4.00	4.00	4.00
N_{Lim}/N_{Total}	0/37	0/37	4/37	15/37	29/37	0/37	0/37	13/37	27/37	34/37

N_{Lim}, number of participants reaching the maximum stimulation intensity threshold (4 mA) in that condition; N_{Total}, total number of participants; *CD*, current density

less perceivable (Hsu et al., 2021; Hutcheon & Yarom, 2000; Turi et al., 2013; Zeng et al., 2019), thus potentially allowing for the application of higher stimulation intensities. In our study, we sought to quantify the sensation and pain thresholds for various high-frequency alternating current stimulations used in tTIS, aiming to explore the potential upper limits of intensity for this innovative electrostimulation technique. Furthermore, we explored the possibility to push these limits by employing an anesthetizing topical cream, to reduce cutaneous sensations caused by electrical stimulation. To be able benchmark the perception of these high frequencies against classical methods of electrostimulation, we've also quantified perception of low-frequency alternating currents as commonly used in tACS.

Our findings are in line with other studies, demonstrating that as stimulation frequency increases, its perceptibility decreases (Hsu et al., 2021; Imatz-Ojanguren & Keller, 2023; Turi et al., 2013; Ward & Robertson, 1998; Zeng et al., 2019). Additionally, consistent with findings from other studies, we verified that application of an anesthetizing cream reduces perceptibility during transcranial electrostimulation (Guleyupoglu et al., 2014; McFadden et al., 2011). The reduced perceptibility of high-frequency stimulation offers an added advantage for ensuring participant blinding, given its increased perception thresholds compared to traditional electrostimulation methods. Moreover, the use of an anesthetizing cream appears to be a valuable tool in increasing participant comfort and blinding.

The included lower frequencies of 10 Hz and 20 Hz in our study reflect standard frequencies employed in tACS studies (Herrmann & Strüber, 2017; Koninck et al., 2023; Wischnewski et al., 2019; Yavari et al., 2018). Our findings demonstrate that these frequencies are already perceptible for most participants at low stimulation intensities, while discomfort or pain became noticeable at slightly higher intensities. In addition, the application of the anesthetizing cream was able to increase these thresholds, proving its effectiveness in reducing somatosensory side effects during stimulation like tingling, itching, or stinging sensations. Furthermore, the higher frequencies of 500 Hz, 1000 Hz, and 2000 Hz selected for this study reflect stimulation frequencies used in current tTIS studies (von Conta et al., 2022; Esmaeilpour et al., 2021; Grossman et al., 2017; Ma et al., 2021; Sunshine et al., 2021; Zhu et al., 2022). Due to reduced sensitivity to higher frequencies in somatosensory perception (Hutcheon & Yarom, 2000; Palmer et al., 1999), we were able to confirm that these frequencies allow for a substantial increase in stimulation intensity before becoming perceptible or inducing pain. In addition, we were able to push the limits of comfortable stimulation intensities even further with the use of anesthetization. Notably, our results indicate that the anesthetic effect seemed to decrease at frequencies of 1000 Hz and above. However, as explained, this is not indicative of reduced anesthetic efficacy, but rather a ceiling effect inherent to our study design. Nonetheless, these results highlight the potential for using high-frequency stimulation in conjunction with topical anesthetization at

increased intensities, without compromising participant blinding or causing discomfort.

Notably, for the higher frequencies, a portion of the participants had their thresholds set to 4 mA for statistical analysis in line with staircase conclusion criterion 2. This indicates that even when the maximum stimulation intensity of 4 mA was reached in a condition, these participants did not report pain or perceivable sensations. This is especially true for stimulation with 2000 Hz, in which 29 out of 37 subjects reached 4 mA. Therefore, it's crucial to recognize that the actual thresholds for these frequencies likely surpass our applied maximum of 4 mA. This limitation is due to the ethical, safety, and hardware constraints within which our study was conducted. Consequently, the recorded thresholds at these frequencies essentially represent the highest stimulation intensities we could safely administer, highlighting that participants might have tolerated even higher intensities during high-frequency stimulation without discomfort. Therefore, our statistical analysis is even on the conservative side and likely underestimates the true tolerable thresholds.

On the other side, some subjects reported pain sensations for high frequencies at considerably lower intensities (e.g., for 2000 Hz, one participant's pain threshold was measured as 2.7 mA). This highlights the interindividual differences in perception to electrostimulation which should be taken into account. Especially for low frequencies, our results reveal considerable variability in pain and perception thresholds among participants. For instance, in the 10 Hz condition, pain thresholds range from 0.18 to 1.78 mA. This highlights that both high- and low-frequency stimulation vary in individual sensitivity and there is no one-size-fits-all approach. Therefore, our thresholds should be viewed as approximate guidelines rather than absolute values. However, our data indicate that even in common tACS studies, it is highly probable that some subjects will perceive the stimulation and few subjects will even feel pain, even if the stimulation is within a safe range of <2 mA. Thus, for optimal safety and successful blinding, we advise testing individual thresholds before applying electrostimulation at the target intensity.

It is essential to recognize that the effectiveness of stimulation is not solely determined by the applied stimulation intensity. While numerous studies suggest a dose-dependent effect, indicating that higher stimulation intensities often lead to more pronounced effects (Johnson et al., 2020; Turner et al., 2021; Wischnewski et al., 2019), this is not an absolute rule. Indeed, some research indicates a complex, non-linear relationship between stimulation intensity and its effects. A study by Moliadze et al. (2012) has shown that while lower intensities might lead to inhibition, increasing the intensity can actually reverse this effect, transforming inhibition into excitation. Moreover, the actual voltage that reaches the target area is not guaranteed by high stimulation intensity alone. Variabilities in individual anatomical factors, such as skull thickness, and the specific configuration of the electrode montage, significantly influence the voltage delivered to the target area (Hunold et al., 2023). Consequently, it is recommended to utilize current flow modeling tools, like SimNIBS (Puonti et al., 2020; Thielscher et al., 2015) or ROAST (Huang et al., 2019). These tools, particularly when modeled on individual anatomical specifics, can be used to ensure that the target area receives sufficient voltage for effective stimulation (Saturnino et al., 2019).

It is important to emphasize that the stimulation thresholds in our study were determined using round electrodes with a diameter of 12 mm. To ensure that our results are relevant irrespective of electrode size, we have included measures of current density alongside our findings. However, it's essential to understand the interplay between electrode size, current density, and current intensity. Several studies have posited that larger electrodes generally offer more comfort than smaller ones, attributed to the distribution of currents across a larger area, leading to lower current densities (Alon et al., 1994; McNeal & Baker, 1988; Verhoeven & van Dijk, 2006). Yet, Lyons et al. (2004) presented contrasting evidence, showing greater comfort with smaller electrodes. This contradiction may be solved by recent research, which suggests that cutaneous sensation is primarily influenced by current intensity rather than current density (Fertonani et al., 2015; Martinsen et al., 2004; Turi et al., 2014). This is due to a spatial summation effect where larger electrodes engage more cutaneous receptors, increasing sensation (Higashiyama, 1993; Higashiyama & Tashiro, 1990; Nielsen & Arendt-Nielsen, 1997). Consequently, our results regarding perception and pain thresholds still provide valuable guidance for studies using larger electrodes. However, it's worth noting that cutaneous sensations can be influenced by other variables, such as the concentration of a saline solution used as a contact medium (Dundas et al., 2007), though they are not affected by the shape of the electrode (Ambrus et al., 2011).

Certainly, safe stimulation intensities cannot be based solely on the lack of immediate cutaneous pain sensations. Research confirms the safety of classical electrostimulation, as evidenced by rodent model studies. These studies show no changes in several neurotoxicity markers for stimulation intensities commonly used in humans (Jackson et al., 2017; Liebetanz et al., 2009; Zhang et al., 2019). This becomes also evident in human studies, which confirm the absence of neurotoxicity or serious adverse effects of stimulation (Nitsche et al., 2003; Nitsche et al., 2004; 2001; Tadini et al., 2011). Currently, stimulation intensities of up to 4 mA are considered safe using electrostimulation methods such as tACS or tDCS (Antal et al., 2017; Bikson et al., 2016; Fertonani et al., 2015; Matsumoto & Ugawa, 2017; Nitsche & Bikson, 2017). Taking into account the novel high-frequency stimulation methods, Grossman et al. (2017) did not find tissue damage in rodents,

and Piao et al. (2022) did not find an adverse effect of this stimulation on various tested criteria in humans. This is in line with Cassarà et al. (2022), who explored the safety of tTIS in humans and recommended frequency-based maximum exposure limits, with higher frequencies allowing for greater exposure. Nonetheless, to increase stimulation intensities above 4 mA, a robust body of evidence pointing to its unquestionable safety is needed.

A limitation of our findings is the brief 5-s duration of stimulation to measure thresholds. Previous research indicates that stimulation sensation decreases over time due to adaptation effects (Hsu et al., 2021; Khadka et al., 2020). This adaptation effect explains why tACS studies can administer intensities of 1 mA or more without causing prolonged discomfort. Consequently, the thresholds identified in our study are likely conservative, potentially underestimating the maximum tolerable stimulation intensities. Employing a procedure where stimulation is applied for an extended duration with a gradual increase could leverage this adaptation effect to even further increase perception and pain thresholds. This would allow for even more intense, yet still comfortable, electrical stimulation. Future research should explore the limits of this adaptive approach for high-frequency alternating current stimulation.

In addition, in our study, we relied on participants' self-reports to determine thresholds, a method that inherently carries the risk of subjective biases. To enhance the robustness of future studies, incorporating objective indicators, like physiological markers of discomfort including skin conductivity (Storm, 2008; Syrjala et al., 2019), might offer a more consistent gauge of participant comfort.

Furthermore, is well-documented that pain perception can be influenced by an individual's physical and psychological state. Factors such as age (Lautenbacher et al., 2017), expectation (Wiech, 2016; Wiech et al., 2008), fatigue (Lautenbacher et al., 2006), or sex (Paller et al., 2009; Wiesenfeld-Hallin, 2005) influence how pain is experienced. This is in line with our study's results, where males were able to tolerate higher stimulation intensities compared with females. However, given that the sample in our study had a bias towards female participants (25 female, 12 male), the generalization of our findings should be done with a degree of caution. A future study systematically comparing sex differences would be needed to substantiate this result. However, we believe that our results serve as a robust foundation for establishing new limits and possibilities for future stimulation studies.

Conclusion

We demonstrated that the somatosensory perception and pain thresholds for alternating current stimulation are frequency-dependent. Utilizing high-frequency stimulation, we successfully administered intensities of up to 4 mA without inducing discomfort in participants. This finding is especially of note for tTIS, whose efficacy has been limited by low-intensity protocols so far. Increasing the stimulation intensity has the potential to enhance the efficacy of tTIS, unlocking the potential for non-invasive stimulation of deeper brain regions. Additionally, the use of topical anesthetic cream further elevates these thresholds, enabling even higher intensities. This finding also translates to tACS applications in general, allowing for more potent neuromodulatory interventions without compromising participant comfort.

In summary, our findings reveal significant interindividual differences in perception and pain thresholds, particularly under high-frequency conditions, emphasizing the need for customized stimulation intensities in tTIS/tACS experiments. To ensure participant comfort and effective blinding, we recommend tailoring stimulation based on individual responses. Additionally, our study shows that using a topical anesthetic can raise these thresholds, offering a viable method to enhance participant tolerance or blinding in future electrostimulation studies.

AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT-4 in order to improve readability and language of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Acknowledgements We thank all members of the Zaehle Lab for their valuable contributions to discussions. We would furthermore like to thank three anonymous reviewers for their helpful comments on an earlier version of this manuscript.

Author Contribution Carsten Thiele: Conceptualization; methodology; software; investigation; formal analysis; data curation; writing, original draft; visualization.

Cornelius Tamm: Investigation, formal analysis, writing—review and editing.

Philipp Ruhnau: Resources; writing, review and editing; supervision; project administration; funding acquisition.

Tino Zaehle: Conceptualization; resources; writing, review and editing; supervision; project administration; funding acquisition.

Funding This study was supported by the federal state of Saxony-Anhalt and the European Regional Development Fund (ERDF) in the Center for Behavioral Brain Sciences (CBBS, ZS/2016/04/78113). The funding source had no direct involvement in this study.

Data Availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

References

Acerbo, E., Jegou, A., Luff, C., Dzialecka, P., Botzanowski, B., Missey, F., Ngom, I., Lagarde, S., Bartolomei, F., Cassara, A., Neufeld, E., Jirsa, V., Carron, R., Grossman, N., & Williamson, A. (2022). Focal non-invasive deep-brain stimulation with temporal interference for the suppression of epileptic biomarkers. *Frontiers in Neuroscience, 16*, 945221.

Alon, G., Kantor, G., & Ho, H. S. (1994). Effects of electrode size on basic excitatory responses and on selected stimulus parameters. *The Journal of Orthopaedic and Sports Physical Therapy, 20*(1), 29–35.

Ambrus, G. G., Paulus, W., & Antal, A. (2010). Cutaneous perception thresholds of electrical stimulation methods: Comparison of tDCS and tRNS. *Clinical Neurophysiology, 121*(11), 1908–1914.

Ambrus, G. G., Antal, A., & Paulus, W. (2011). Comparing cutaneous perception induced by electrical stimulation using rectangular and round shaped electrodes. *Clinical Neurophysiology Official Journal of the International Federation of Clinical Neurophysiology, 122*(4), 803–807.

Anderson, A. B., & Munson, W. A. (1951). Electrical excitation of nerves in the skin at audiofrequencies. *The Journal of the Acoustical Society of America, 23*(2), 155–159.

Antal, A., & Paulus, W. (2013). Transcranial alternating current stimulation (tACS). *Frontiers in Human Neuroscience, 7*, 317.

Antal, A., Alekseichuk, I., Bikson, M., Brockmöller, J., Brunoni, A. R., Chen, R., Cohen, L. G., Dowthwaite, G., Ellrich, J., Flöel, A., Fregni, F., George, M. S., Hamilton, R., Haueisen, J., Herrmann, C. S., Hummel, F. C., Lefaucheur, J. P., Liebetanz, D., Loo, C. K., … Paulus, W. (2017). Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. *Clinical Neurophysiology, 128*(9), 1774–1809.

Arul-Anandam, A. P., & Loo, C. (2009). Transcranial direct current stimulation: A new tool for the treatment of depression? *Journal of Affective Disorders, 117*(3), 137–145.

Asamoah, B., Khatoun, A., & Mc, L. M. (2019). tACS motor system effects can be caused by transcutaneous stimulation of peripheral nerves. *Nature Communications, 10*(1), 266.

Baker, L. L., Bowman, B. R., & McNeal. (1988). Effects of waveform on comfort during neuromuscular electrical stimulation. *Clinical Orthopaedics and Related Research, 233*, 75–85.

Bikson, M., Datta, A., & Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clinical Neurophysiology, 120*(6), 1033–1034.

Bikson, M., Grossman, P., Thomas, C., Zannou, A. L., Jiang, J., Adnan, T., Mourdoukoutas, A. P., Kronberg, G., Truong, D., Boggio, P., Brunoni, A. R., Charvet, L., Fregni, F., Fritsch, B., Gillick, B., Hamilton, R. H., Hampstead, B. M., Jankord, R., Kirton, A., … Woods, A. J. (2016). Safety of transcranial direct current stimulation: Evidence based update 2016. *Brain Stimulation, 9*(5), 641–661.

Budde, R. B., Williams, M. T., & Irazoqui. (2023). Temporal interference current stimulation in peripheral nerves is not driven by envelope extraction. *Journal of Neural Engineering, 20*(2), 026041.

Cao J, ron B, Goswami C, and Grover P. The mechanics of temporal interference stimulation. *bioRxiv*: 2020.04.23.051870, 2020.

Cao J. 2018 Do single neuron models exhibit temporal interference stimulation?, 2018 Cao J, Grover P. Do single neuron models exhibit temporal interference stimulation?. In2018 IEEE Biomedical Circuits and Systems Conference (BioCAS) 2018 Oct 17 (pp. 1-4). IEEE

Carmona-Barrón VG, Fernández del Campo IS, Delgado-García JM, De la Fuente AJ, Lopez IP, Merchán MA. 2023 Comparing the effects of transcranial alternating current and temporal interference (tTIS) electric stimulation through whole-brain mapping of c-Fos immunoreactivity. Frontiers in Neuroanatomy 17 1128193

Chang, S. (2022). The application of transcranial electrical stimulation in sports psychology. *Computational and Mathematical Methods in Medicine, 2022*, 1008346.

Chhatbar, P. Y., Chen, R., Deardorff, R., Dellenbach, B., Kautz, S. A., George, M. S., & Feng, W. (2017). Safety and tolerability of transcranial direct current stimulation to stroke patients - A phase I current escalation study. *Brain Stimulation, 10*(3), 553–559.

Cho, H., Razza, L. B., Borrione, L., Bikson, M., Charvet, L., Dennis-Tiwary, T. A., Brunoni, A. R., & Sudbrack-Oliveira, P. (2022). Transcranial electrical stimulation for psychiatric disorders in adults: A primer. *Focus, 20*(1), 19–31.

Convento, S., Russo, C., Zigiotto, L., & Bolognini, N. (2016). Transcranial electrical stimulation in post-stroke cognitive rehabilitation. *European Psychologist, 21*(1), 55–64.

Cornsweet, T. N. (1962). The staircase-method in psychophysics. *The American Journal of Psychology, 75*(3), 485–491.

De Koninck, B. P., Brazeau, D., Guay, S., Babiloni, A. H., & De Beaumont, L. (2023). Transcranial alternating current stimulation to modulate alpha activity: A systematic review. *Neuromodulation: Technology at the Neural Interface, 26*(8), 1549–84.

Dundas, J. E., Thickbroom, G. W., & Mastaglia, F. L. (2007). Perception of comfort during transcranial DC stimulation: Effect of NaCl solution concentration applied to sponge electrodes. *Clinical Neurophysiology, 118*(5), 1166–1170.

Esmaeilpour, Z., Kronberg, G., Reato, D., Parra, L. C., & Bikson, M. (2021). Temporal interference stimulation targets deep brain regions by modulating neural oscillations. *Brain Stimulation, 14*(1), 55–65.

Fertonani, A., Ferrari, C., & Miniussi, C. (2015). What do you feel if I apply transcranial electric stimulation? Safety, sensations and secondary induced effects. *Clinical Neurophysiology, 126*(11), 2181–2188.

Friehs, M. A., Whelan, E., Güldenpenning, I., Krause, D., & Weigelt, M. (2022). Stimulating performance: A scoping review on transcranial electrical stimulation effects on Olympic sports. *Psychology of Sport and Exercise, 59*, 102130.

Grossman, N., Bono, D., Dedic, N., Kodandaramaiah, S. B., Rudenko, A., Suk, H.-J., Cassara, A. M., Neufeld, E., Kuster, N., & Tsai, L.-H. (2017). Noninvasive deep brain stimulation via temporally interfering electric fields. *Cell, 169*(6), 1029–1041.

Grossman, N., Okun, M. S., & Boyden, E. S. (2018). Translating temporal interference brain stimulation to treat neurological and psychiatric conditions. *JAMA Neurology, 75*(11), 1307–1308.

Guleyupoglu, B., Febles, N., Minhas, P., Hahn, C., & Bikson, M. (2014). Reduced discomfort during high-definition

transcutaneous stimulation using 6% benzocaine. *Frontiers in Neuroengineering, 7*, 28.
Hawkes, G. R., & Warm, J. S. (1960). The sensory range of electrical stimulation of the skin. *The American Journal of Psychology, 73*(3), 485.
He, Q., Yang, X.-Y., Zhao, D., & Fang, F. (2022). Enhancement of visual perception by combining transcranial electrical stimulation and visual perceptual training. *Medical Review, 2*(3), 271–284.
Herrmann, C. S., & Strüber, D. (2017). What can transcranial alternating current stimulation tell us about brain oscillations? *Current Behavioral Neuroscience Reports, 4*(2), 128–137.
Higashiyama, A. (1993). Electrocutaneous spatial integration at suprathreshold levels: An additive neural model. *Journal of Experimental Psychology: Human Perception and Performance, 19*(4), 912–923.
Higashiyama, A., & Tashiro, T. (1990). Electrocutaneous spatial integration at threshold: The effects of electrode size. *Perception & Psychophysics, 48*(4), 389–397.
Hsu, G., Farahani, F., & Parra, L. C. (2021). Cutaneous sensation of electrical stimulation waveforms. *Brain Stimulation, 14*(3), 693–702.
Hsu, G., Shereen, A. D., Cohen, L. G., & Parra, L. C. (2023). Robust enhancement of motor sequence learning with 4 mA transcranial electric stimulation. *Brain Stimulation, 16*(1), 56–67.
Huang, Y., Datta, A., Bikson, M., & Parra, L. C. (2019). Realistic volumetric-approach to simulate transcranial electric stimulation-ROAST-A fully automated open-source pipeline. *Journal of Neural Engineering, 16*(5), 56006.
Hunold, A., Haueisen, J., Nees, F., & Moliadze, V. (2023). Review of individualized current flow modeling studies for transcranial electrical stimulation. *Journal of Neuroscience Research, 101*(4), 405–423.
Hutcheon, B., & Yarom, Y. (2000). Resonance, oscillation and the intrinsic frequency preferences of neurons. *Trends in Neurosciences, 23*(5), 216–222.
Imatz-Ojanguren, E., & Keller, T. (2023). Evoked sensations with transcutaneous electrical stimulation with different frequencies, waveforms, and electrode configurations. *Artificial Organs, 47*(1), 117–128.
Iszak, K., Gronemann, S. M., Meyer, S., Hunold, A., Zschüntzsch, J., Bähr, M., Paulus, W., & Antal, A. (2023). Why temporal inference stimulation may fail in the human brain: A pilot research study. *Biomedicines, 11*(7), 2023.
Jackson, M. P., Truong, D., Brownlow, M. L., Wagner, J. A., McKinley, R. A., Bikson, M., & Jankord, R. (2017). Safety parameter considerations of anodal transcranial direct current stimulation in rats. *Brain, Behavior, and Immunity, 64*, 152–161.
Johnson, L., Alekseichuk, I., Krieg, J., Doyle, A., Yu, Y., Vitek, J., Johnson, M., & Opitz, A. (2020). Dose-dependent effects of transcranial alternating current stimulation on spike timing in awake nonhuman primates. *Science Advances, 6*(36), eaaz2747.
Karimi, F., Attarpour, A., Amirfattahi, R., & Nezhad, A. Z. (2019). Computational analysis of non-invasive deep brain stimulation based on interfering electric fields. *Physics in Medicine and Biology, 64*(23), 235010.
Khadka, N., Borges, H., Paneri, B., Kaufman, T., Nassis, E., Zannou, A. L., Shin, Y., Choi, H., Kim, S., Lee, K., & Bikson, M. (2020). Adaptive current tDCS up to 4 mA. *Brain Stimulation, 13*(1), 69–79.
Khan, A., Yuan, K., Bao, S.-C., Ti, C. H. E., Tariq, A., Anjum, N., & Tong, R.K.-Y. (2022). Can transcranial electrical stimulation facilitate post-stroke cognitive rehabilitation? A systematic review and meta-analysis. *Frontiers in Rehabilitation Sciences, 3*, 795737.
Kleiner M, Brainard D, and Pelli D. 2007 What's new in Psychtoolbox-3?
Koganemaru, S., Mikami, Y., Matsuhashi, M., Truong, D. Q., Bikson, M., Kansaku, K., & Mima, T. (2020). Cerebellar transcranial alternating current stimulation modulates human gait rhythm. *Neuroscience Research, 156*, 265–270.
Kuhn, A., Keller, T., Lawrence, M., & Morari, M. (2010). The influence of electrode size on selectivity and comfort in transcutaneous electrical stimulation of the forearm. *IEEE Transactions on Neural Systems and Rehabilitation Engineering, 18*(3), 255.
Lautenbacher, S., Kundermann, B., & Krieg, J.-C. (2006). Sleep deprivation and pain perception. *Sleep Medicine Reviews, 10*(5), 357–369.
Lautenbacher, S., Peters, J. H., Heesen, M., Scheel, J., & Kunz, M. (2017). Age changes in pain perception: A systematic-review and meta-analysis of age effects on pain and tolerance thresholds. *Neuroscience and Biobehavioral Reviews, 75*, 104–113.
Leek, M. R. (2001). Adaptive procedures in psychophysical research. *Perception & Psychophysics, 63*(8), 1279–1292.
Liebetanz, D., Koch, R., Mayenfels, S., König, F., Paulus, W., & Nitsche, M. A. (2009). Safety limits of cathodal transcranial direct current stimulation in rats. *Clinical Neurophysiology Official Journal of the International Federation of Clinical Neurophysiology, 120*(6), 1161–1167.
Linnhoff, S., Fiene, M., Heinze, H.-J., & Zaehle, T. (2019). Cognitive fatigue in multiple sclerosis: An objective approach to diagnosis and treatment by transcranial electrical stimulation. *Brain Sciences, 9*(5), 100.
Liu, A., Vöröslakos, M., Kronberg, G., Henin, S., Krause, M. R., Huang, Y., Opitz, A., Mehta, A., Pack, C. C., Krekelberg, B., Berényi, A., Parra, L. C., Melloni, L., Devinsky, O., & Buzsáki, G. (2018). Immediate neurophysiological effects of transcranial electrical stimulation. *Nature Communications, 9*(1), 5092.
Liu, X., Qi, S., Hou, L., Liu, Y., & Wang, X. (2024). Noninvasive deep brain stimulation via temporal interference electric fields enhanced motor performance of mice and its neuroplasticity mechanisms. *Molecular Neurobiology, 61*(6), 3314–29.
Lyons, G. M., Leane, G. E., Clarke-Moloney, M., O'Brien, J. V., & Grace, P. A. (2004). An investigation of the effect of electrode size and electrode location on comfort during stimulation of the gastrocnemius muscle. *Medical Engineering & Physics, 26*(10), 873–878.
Ma, R., Xia, X., Zhang, W., Lu, Z., Wu, Q., Cui, J., Song, H., Fan, C., Chen, X., & Zha, R. (2021). High gamma and beta temporal interference stimulation in the human motor cortex improves motor functions. *Frontiers in Neuroscience, 15*, 800436.
Martinez, S. C., Goswami, C., Forssell, M., Cao, J., Barth, A., & Grover, P. (2023). Cell-specific effects of temporal interference stimulation on cortical function. *Research Square*, PREPRINT (Version 1). https://doi.org/10.21203/rs.3.rs-2395375/v1
Martinsen, Ø. G., Grimnes, S., & Piltan, H. (2004). Cutaneous perception of electrical direct current. *ITBM-RBM, 25*(4), 240–243.
Matsumoto, H., & Ugawa, Y. (2017). Adverse events of tDCS and tACS: A review. *Clinical Neurophysiology Practice, 2*, 19–25.
McFadden, J. L., Borckardt, J. J., George, M. S., & Beam, W. (2011). Reducing procedural pain and discomfort associated with transcranial direct current stimulation. *Brain Stimulation, 4*(1), 38–42.
McNeal, D. R., & Baker, L. L. (1988). Effects of joint angle, electrodes and waveform on electrical stimulation of the quadriceps and hamstrings. *Annals of Biomedical Engineering, 16*(3), 299–310.
Mirzakhalili, E., Barra, B., Capogrosso, M., & Lempka, S. F. (2020). Biophysics of temporal interference stimulation. *Cell Systems, 11*(6), 557-572.e5.
Missey, F., Rusina, E., Acerbo, E., Botzanowski, B., Trébuchon, A., Bartolomei, F., Jirsa, V., Carron, R., & Williamson, A. (2021). Orientation of temporal interference for non-invasive deep brain stimulation in epilepsy. *Frontiers in Neuroscience, 15*, 633988.

Moliadze, V., Atalay, D., Antal, A., & Paulus, W. (2012). Close to threshold transcranial electrical stimulation preferentially activates inhibitory networks before switching to excitation with higher intensities. *Brain Stimulation, 5*(4), 505–511.

Negahbani, E., Kasten, F. H., Herrmann, C. S., & Fröhlich, F. (2018). Targeting alpha-band oscillations in a cortical model with amplitude-modulated high-frequency transcranial electric stimulation. *NeuroImage, 173*, 3–12.

Nielsen, J., & Arendt-Nielsen, L. (1997). Spatial summation of heat induced pain within and between dermatomes. *Somatosensory & Motor Research, 14*(2), 119–125.

Nitsche, M. A., & Bikson, M. (2017). Extending the parameter range for tDCS: Safety and tolerability of 4 mA stimulation. *Brain Stimulation, 10*(3), 541–542.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*(10), 1899–1901.

Nitsche, M. A., Nitsche, M. S., Klein, C. C., Tergau, F., Rothwell, J. C., & Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clinical Neurophysiology, 114*(4), 600–604.

Nitsche, M. A., Niehaus, L., Hoffmann, K. T., Hengst, S., Liebetanz, D., Paulus, W., & Meyer, B.-U. (2004). MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clinical Neurophysiology, 115*(10), 2419–2423.

Paller, C. J., Campbell, C. M., Edwards, R. R., & Dobs, A. S. (2009). Sex-based differences in pain perception and treatment. *Pain Medicine, 10*(2), 289–299.

Palmer, S. T., Martin, D. J., Steedman, W. M., & Ravey, J. (1999). Alteration of interferential current and transcutaneous electrical nerve stimulation frequency: Effects on nerve excitation. *Archives of Physical Medicine and Rehabilitation, 80*(9), 1065–1071.

Paneri, B., Adair, D., Thomas, C., Khadka, N., Patel, V., Tyler, W. J., Parra, L., & Bikson, M. (2016). Tolerability of repeated application of transcranial electrical stimulation with limited outputs to healthy subjects. *Brain Stimulation, 9*(5), 740–754.

Perrey, S. (2023). Probing the promises of noninvasive transcranial electrical stimulation for boosting mental performance in sports. *Brain Sciences, 13*(2), 282.

Piao, Y., Ma, R., Weng, Y., Fan, C., Xia, X., Zhang, W., Zeng, G. Q., Wang, Y., Lu, Z., Cui, J., Wang, X., Gao, L., Qiu, B., & Zhang, X. (2022). Safety evaluation of employing temporal interference transcranial alternating current stimulation in human studies. *Brain Sciences, 12*(9), 1194.

Puonti, O., van Leemput, K., Saturnino, G. B., Siebner, H. R., Madsen, K. H., & Thielscher, A. (2020). Accurate and robust whole-head segmentation from magnetic resonance images for individualized head modeling. *NeuroImage, 219*, 117044.

Röhner, F., Breitling, C., Rufener, K. S., Heinze, H.-J., Hinrichs, H., Krauel, K., & Sweeney-Reed, C. M. (2018). Modulation of working memory using transcranial electrical stimulation: A direct comparison between TACS and TDCS. *Frontiers in Neuroscience, 12*, 761.

Saturnino, G. B., Siebner, H. R., Thielscher, A., & Madsen, K. H. (2019). Accessibility of cortical regions to focal TES: Dependence on spatial position, safety, and practical constraints. *NeuroImage, 203*, 116183.

Sheffield, J. G., Ramerpresad, S., Brem, A.-K., Mansfield, K., Orhan, U., Dillard, M., McKanna, J., Plessow, F., Thompson, T., Santarnecchi, E., Pascual-Leone, A., Pavel, M., Mathan, S., & Cohen, K. R. (2022). Blinding efficacy and adverse events following repeated transcranial alternating current, direct current, and random noise stimulation. *Cortex, 154*, 77–88.

Shirvani, S., Davoudi, M., Shirvani, M., Koleini, P., Hojat Panah, S., Shoshtari, F., & Omidi, A. (2021). Comparison of the effects of transcranial direct current stimulation and mindfulness-based stress reduction on mental fatigue, quality of life and aggression in mild traumatic brain injury patients: A randomized clinical trial. *Annals of General Psychiatry, 20*(1), 33.

Solomons, C. D., & Shanmugasundaram, V. (2019). A review of transcranial electrical stimulation methods in stroke rehabilitation. *Neurology India, 67*(2), 417–423.

Song, X., Zhao, X., Li, X., Liu, S., & Ming, D. (2021). Multi-channel transcranial temporally interfering stimulation (tTIS): Application to living mice brain. *Journal of Neural Engineering, 18*(3), 036003.

Song S, Zhang J, Tian Y, Wang L, and Wei P. Temporal interference stimulation regulates eye movements and neural activity in the mice superior colliculus. *43rd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)*, 2021: 6231–6234, 2021a.

Song. Typical electrode configuration analysis for temporally interfering deep brain stimulation. *9th International IEEE/EMBS* Conference *on Neural Engineering (NER)*: 770–773, 2019.

Spooner, R. K., Wiesman, A. I., & Wilson, T. W. (2022). Peripheral somatosensory entrainment modulates the cross-frequency coupling of movement-related theta-gamma oscillations. *Brain Connectivity, 12*(6), 524–537.

Storm, H. (2008). Changes in skin conductance as a tool to monitor nociceptive stimulation and pain. *Current Opinion in Anaesthesiology, 21*(6), 796–804.

Sunshine, M. D., Cassarà, A. M., Neufeld, E., Grossman, N., Mareci, T. H., Otto, K. J., Boyden, E. S., & Fuller, D. D. (2021). Restoration of breathing after opioid overdose and spinal cord injury using temporal interference stimulation. *Communications Biology, 4*(1), 107.

Syrjala E, Jiang M, Pahikkala T, Salantera S, and Liljeberg P. Skin conductance response to gradual-increasing experimental pain. In *2019 41st Annual International Conference of the IEEE Engineering in Medicine and Biology* Society *(EMBC)*: IEEE, 2019.

Tadini, L., El-Nazer, R., Brunoni, A. R., Williams, J., Carvas, M., Boggio, P., Priori, A., Pascual-Leone, A., & Fregni, F. (2011). Cognitive, mood, and electroencephalographic effects of noninvasive cortical stimulation with weak electrical currents. *The Journal of ECT, 27*(2), 134–140.

Thielscher A, Antunes A, and Saturnino GB. Field modeling for transcranial magnetic stimulation: a useful tool to understand the physiological effects of TMS? *37th annual international conference of* the *IEEE engineering in medicine and biology society (EMBC)*, 2015.

Turi, Z., Ambrus, G. G., Janacsek, K., Emmert, K., Hahn, L., Paulus, W., & Antal, A. (2013). Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation. *Restorative Neurology and Neuroscience, 31*(3), 275–285.

Turi, Z., Ambrus, G. G., Ho, K.-A., Sengupta, T., Paulus, W., & Antal, A. (2014). When size matters: Large electrodes induce greater stimulation-related cutaneous discomfort than smaller electrodes at equivalent current density. *Brain stimulation, 7*(3), 460–467.

Turi Z, Mittner M, Lehr A, Bürger H, Antal A, and Paulus W. 2020 θ-γ cross-frequency transcranial alternating current stimulation over the trough impairs cognitive control. *Eneuro* 7 (5)

Turner, D. A., Degan, S., Galeffi, F., Schmidt, S., & Peterchev, A. V. (2021). Rapid, Dose-dependent enhancement of cerebral blood flow by transcranial AC stimulation in mouse. *Brain Stimulation, 14*(1), 80–87.

Vanderhasselt, M.-A., de Raedt, R., Namur, V., Lotufo, P. A., Bensenor, I. M., Boggio, P. S., & Brunoni, A. R. (2015). Transcranial electric stimulation and neurocognitive training in clinically depressed patients: A pilot study of the effects on rumination.

Progress in Neuro-Psychopharmacology & Biological Psychiatry, 57, 93–99.

Verhoeven, K., & van Dijk, J. G. (2006). Decreasing pain in electrical nerve stimulation. *Clinical Neurophysiology, 117*(5), 972–978.

von Conta, J., Kasten, F. H., Schellhorn, K., Ćurčić-Blake, B., Aleman, A., & Herrmann, C. S. (2022). Benchmarking the effects of transcranial temporal interference stimulation (tTIS) in humans. *Cortex, 154*, 299–310.

Wang, Y., Shi, L., Dong, G., Zhang, Z., & Chen, R. (2020). Effects of transcranial electrical stimulation on human auditory processing and behavior-A review. *Brain Sciences, 10*(8), 531.

Wang, B., Aberra, A. S., Grill, W. M., & Peterchev, A. V. (2023). Responses of model cortical neurons to temporal interference stimulation and related transcranial alternating current stimulation modalities. *Journal of Neural Engineering, 19*(6), 66047.

Ward, A. R., & Robertson, V. J. (1998). Sensory, motor, and pain thresholds for stimulation with medium frequency alternating current. *Archives of Physical Medicine and Rehabilitation, 79*(3), 273–278.

Wiech, K. (2016). Deconstructing the sensation of pain: The influence of cognitive processes on pain perception. *Science, 354*(6312), 584–587.

Wiech, K., Ploner, M., & Tracey, I. (2008). Neurocognitive aspects of pain perception. *Trends in Cognitive Sciences, 12*(8), 306–313.

Wiesenfeld-Hallin, Z. (2005). Sex differences in pain perception. *Gender Medicine, 2*(3), 137–145.

Wischnewski, M., Schutter, D. J. L. G., & Nitsche, M. A. (2019). Effects of beta-tACS on corticospinal excitability: A meta-analysis. *Brain Stimulation, 12*(6), 1381–1389.

Yavari, F., Jamil, A., Mosayebi Samani, M., Vidor, L. P., & Nitsche, M. A. (2018). Basic and functional effects of transcranial electrical stimulation (tES)-An introduction. *Neuroscience and Biobehavioral Reviews, 85*, 81–92.

Zaehle, T., Sandmann, P., Thorne, J. D., Jäncke, L., & Herrmann, C. S. (2011). Transcranial direct current stimulation of the prefrontal cortex modulates working memory performance: Combined behavioural and electrophysiological evidence. *BMC Neuroscience, 12*(1), 2.

Zeng, F.-G., Tran, P., Richardson, M., Sun, S., & Xu, Y. (2019). Human sensation of transcranial electric stimulation. *Scientific Reports, 9*(1), 1–12.

Zhang, K., Guo, L., Zhang, J., An, G., Zhou, Y., Lin, J., Xing, J., Lu, M., & Ding, G. (2019). A safety study of 500 μA cathodal transcranial direct current stimulation in rat. *BMC Neuroscience, 20*(1), 40.

Zhang, Z., Lin, B.-S., Wu, C.-W.G., Hsieh, T.-H., Liou, J.-C., Li, Y.-T., & Peng, C.-W. (2022). Designing and pilot testing a novel transcranial temporal interference stimulation device for neuromodulation. *IEEE Transactions on Neural Systems and Rehabilitation Engineering, 30*, 1483–1493.

Zhu, Z., Xiong, Y., Chen, Y., Jiang, Y., Qian, Z., Lu, J., Liu, Y., & Zhuang, J. (2022). Temporal interference (TI) stimulation boosts functional connectivity in human motor cortex: A comparison study with transcranial direct current stimulation (tDCS). *Neural Plasticity, 2022*, 7605046.